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STIC Database Tracking Number: 100562

To: Gary Counts **Location:** CM1-7E12 **Art Unit:** 1641 **Friday, August 08, 2003** **Case Serial Number:** 09/932369	**From:** Beverly Shears **Location:** Biotech-Chem Library CM1-1E05 **Phone:** 308-4994 beverly.shears@uspto.gov

Search Notes

Counts, G.
09/932369


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     FILE 'REGISTRY' ENTERED AT 09:03:33 ON 08 AUG 2003
L1             4 S ("OXYGEN-18" OR "NITROGEN-15" OR "CARBON-13" OR "HYDROG
L2             2 S (TRYPSIN OR PROTEOLYTIC ENZYME)/CN

     FILE 'HCAPLUS' ENTERED AT 09:04:06 ON 08 AUG 2003
L1             4 SEA FILE=REGISTRY ABB=ON  PLU=ON  ("OXYGEN-18" OR
                 "NITROGEN-15" OR "CARBON-13" OR "HYDROGEN-2")/CN
L2             2 SEA FILE=REGISTRY ABB=ON  PLU=ON  (TRYPSIN OR PROTEOLYTIC
                  ENZYME)/CN
L3        313806 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L1 OR 18O OR 15N OR 13C
                 OR 2H OR OXYGEN(1A)18 OR NITROGEN(1A)15 OR CARBON(1A)13
                 OR HYDROGEN(1A)2 OR OXYGEN18 OR 18OXYGEN OR NITROGEN15
                 OR 15NITROGEN OR 13CARBON OR CARBON13 OR 2HYDROGEN OR
                 HYDROGEN2 OR DEUTERIUM
L4           502 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 AND (L2 OR PROTEOLYTI
                 C ENZYME OR TRYPSIN)
L5           343 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L4 AND (BIOPOLYMER OR
                 BIOMOL OR BIOMOLECULE OR BIO(W)(POLYMER OR MOL OR
                 MOLECULE) OR PROTEIN OR PEPTIDE OR POLYPROTEIN OR
                 POLYPEPTIDE OR ANTIGEN OR ANTIBOD?)
L6            58 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L5 AND (MASS SPECTROMET?
                  OR MS(S)SPECTROMET? OR SPECTRUM ANALYSIS)
L7            29 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L6 AND (RESOLV? OR
                 SEPARAT? OR SEP## OR HPLC OR (LIQ OR LIQUID)(W)CHROMATOGR
                 ? OR ELECTROPHOR?)

L7   ANSWER 1 OF 29  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:        2003:496535  HCAPLUS
TITLE:                   Trypsin catalyzed 16O-to-18O
                         exchange for comparative proteomics: tandem
                         mass spectrometry comparison
                         using MALDI-TOF, ESI-QTOF, and ESI-ion trap
                         mass spectrometers
AUTHOR(S):               Heller, Manfred; Mattou, Hassan; Menzel,
                         Christoph; Yao, Xudong
CORPORATE SOURCE:        GeneProt Inc., Meyrin, Switz.
SOURCE:                  Journal of the American Society for Mass
                         Spectrometry (2003), 14(7), 704-718
                         CODEN: JAMSEF; ISSN: 1044-0305
PUBLISHER:               Elsevier Science Inc.
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   Quant. or comparative proteome anal. was initially performed with
     2-dimensional gel electrophoresis with the inherent
     disadvantages of being biased towards certain proteins and
     being labor intensive.  Alternative mass
     spectrometry-based approaches in conjunction with gel-free
     protein/peptide sepn. have been
     developed in recent years using various stable isotope labeling
     techniques.  Common to all these techniques is the incorporation,
     biosynthetically or chem., of a labeling moiety having either a
     natural isotope distribution of hydrogen, carbon, oxygen, or
     nitrogen (light form) or being enriched with heavy isotopes like
     deuterium, 13C, 18O, or 15N,
     resp.  By mixing equal amts. of a control sample possessing for
     instance the light form of the label with a heavy-labeled case
     sample, differentially labeled peptides are detected by
     mass spectrometric methods and their intensities
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date rd 5/08 (BD)

serve as a means for direct relative **protein** quantification. While each of the different labeling methods has its advantages and disadvantages, the endoprotease 16O-to-**18O** catalyzed oxygen exchange at the C-terminal carboxylic acid is extremely promising because of the specificity assured by the enzymic reaction and the labeling of essentially every protease-derived **peptide**. We show here that this methodol. is applicable to complex biol. samples such as a subfraction of human plasma. Furthermore, despite the relatively small mass difference of 4 Da between the two labeled forms, corresponding to the exchange of two oxygen atoms by two **18O** isotopes, it is possible to quantify differentially labeled **proteins** on an ion trap **mass spectrometer** with a mass resoln. of about 2000 in automated data dependent LC-**MS/MS** acquisition mode. Post column sample deposition on a MALDI target parallel to online ESI-MS/MS enables the anal. of the same compds. by means of ESI- and MALDI-MS/MS. This has the potential to increase the confidence in the quantification results as well as to increase the sequence coverage of potentially interesting **proteins** by complementary **peptide** ionization techniques. Addnl. the paired y-ion signals in tandem mass spectra of 16O/**18O**-labeled **peptide** pairs provide a means to confirm automatic **protein** identification results or even to assist de novo sequencing of yet unknown **proteins**.

L7 ANSWER 2 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:98177 HCAPLUS
DOCUMENT NUMBER: 138:234255
TITLE: Fast-response proteomics by accelerated in-gel digestion of **proteins**
AUTHOR(S): Havlis, Jan; Thomas, Henrik; Sebela, Marek; Shevchenko, Andrej
CORPORATE SOURCE: Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, D-01307, Germany (BD)
SOURCE: Analytical Chemistry (2003), 75(6), 1300-1306
CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Kinetics of in-gel digestion of **proteins** by modified and native **trypsins** was studied by MALDI TOF **mass spectrometry** using **18O**-labeled **peptides** as internal stds. The effect of the temp., enzyme concn., digestion time, and surface area of gel pieces on the yield of digestion products was characterized. Based on the kinetic data, we developed a protocol that enabled the identification of gel-**sepd**. **proteins** with 30-min digestion time without compromising the **peptide** yield and the sensitivity compared to conventional protocols that typically rely upon overnight enzymic cleavage. The accelerated digestion protocol was tested in identification of more than 120 **proteins** from budding and fission yeasts at the subpicomole level.
IT **9002-07-7, Trypsin**
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(fast-response proteomics by accelerated in-gel digestion of **proteins**)
REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE

FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:41763 HCAPLUS
DOCUMENT NUMBER: 138:350607
TITLE: Use of a lectin affinity selector in the search for unusual glycosylation in proteomics
AUTHOR(S): Xiong, Li; Regnier, Fred E.
CORPORATE SOURCE: Department of Chemistry, Purdue University, West Lafayette, IN, 47907, USA
SOURCE: Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2002), 782(1-2), 405-418
CODEN: JCBAAI; ISSN: 1570-0232
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The purpose of the work described in this paper was to develop a new approach to the identification of glycoprotein with particular types of glycosylation. The paper demonstrates N-glycosylation sites in a glycoproteins can be identified by (1) proteolysis with **trypsin**, (2) lectin affinity selection, (3) enzymic deglycosylation with **peptide**-N-glycosidase F (PNGase F) in buffer contg. 95% H218O, which generates deglycosylated **peptide** pairs **sepd**., by 2 or 4 amu, (4) reversed-phase **sepn**. of the **peptide** mixt. and MALDI mass anal., (5) MS-MS sequencing of the ion pairs, and (6) identification of the parent **protein** through a database search. This process has been tested on the selection of glycopeptides from lactoferrin and mammaglobin, and the identification of the ion pairs of fetuin glycopeptides. Glycosylation sites were identified through PNGase hydrolysis in H218O. During the process of hydrolyzing the conjugate, Asn is converted to an aspartate residue with the incorporation of **18O**. However, PNGase F was obsd. to incorporate two **18O** into the .beta.-carboxyl groups of the Asp residue. This suggests that the hydrolysis is at least partially reversible.

IT **9002-07-7, Trypsin**
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(proteolysis with TPCK-treated **trypsin**; use of lectin affinity selector in search for unusual glycosylation in proteomics)

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:943055 HCAPLUS
DOCUMENT NUMBER: 138:183345
TITLE: Properties of **13C**-substituted arginine in stable isotope labeling by amino acids in cell culture (SILAC)
AUTHOR(S): Ong, Shao-En; Kratchmarova, Irina; Mann, Matthias
CORPORATE SOURCE: Center for Experimental BioInformatics (CEBI) Department of Biochemistry and Molecular

Biology, University of Southern Denmark, Odense, DK-5230, Den.
SOURCE: Journal of Proteome Research (2003), 2(2), 173-181
CODEN: JPROBS; ISSN: 1535-3893
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have recently described a method, stable isotope labeling by amino acids in cell culture (SILAC) for the accurate quantitation of relative **protein** abundances. Cells were metabolically labeled with deuterated leucine, leading to complete incorporation within about five cell doublings. Here, we investigate fully substituted **13C**-labeled arginine in the SILAC method. After tryptic digestion, there is a single label at the C-terminal position in half of the **peptides**. Labeled and unlabeled **peptides** coelute in liq. chromatog.-**mass spectrometric** anal., eliminating quantitation error due to unequal sampling of ion profiles. Tandem mass spectrum interpretation and database identification are aided by the predictable shift of the y-ions in the labeled form. The quantitation of mixts. of total cell lysates in known ratios **resolved** on a one-dimensional SDS-PAGE gel produced consistent and reproducible results with relative std. deviations better than five percent under optimal conditions.
IT **9002-07-7, Trypsin**
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(properties of **13C**-substituted arginine in stable isotope labeling by amino acids in cell culture (SILAC))
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:937755 HCAPLUS
DOCUMENT NUMBER: 138:183318
TITLE: Dissection of proteolytic **18O** labeling: Endoprotease-catalyzed 16O-to-**18O** exchange of truncated **peptide** substrates
AUTHOR(S): Yao, Xudong; Afonso, Carlos; Fenselau, Catherine
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20742, USA
SOURCE: Journal of Proteome Research (2003), 2(2), 147-152
CODEN: JPROBS; ISSN: 1535-3893
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Proteolytic labeling in H218O has been recently revived as a versatile method for proteomics research. To understand the mol. basis of the labeling process, we have dissected the process into two **sep.** events: cleavage of the **peptide** amide bonds and exchange of the terminal carboxyl oxygens. It was demonstrated that both carboxyl oxygens can be catalytically labeled, independent of the cleavage step. Reaction kinetics of the tryptic 16O-to-**18O** exchange of YGGFMR, YGGFMK, and the

tryptic digest of apomyoglobin were studied by matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance **mass spectrometry**. A larger KM for the Lys-**peptide** (4400.+-.700 .mu.M), when compared to that of the Arg-**peptide** (KM 1300.+-.300 .mu.M), was mainly responsible for the slower reaction with YGGFMK (kcat/KM 0.64.+-.0.14 .mu.M-1min-1) compared to YGGFMR (kcat/KM 2.6.+-.0.9 .mu.M-1min-1). Multiplexed kinetic studies showed that endoprotease-catalyzed oxygen exchange is a general phenomenon, allowing homogeneous 18O2-coding of a variety of **peptides**. It was demonstrated for the first time that chymotrypsin 18O2-codes **peptides** during proteolysis. On the basis of the analyses reported here, we propose that proteolytic **18O** labeling can be advantageously decoupled from **protein** digestion, and endoproteases can be used in a **sep**. step to 18O2-code **peptides** for comparative studies after proteolysis has taken place.

IT **14797-71-8, Oxygen-18**, analysis
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(dissection of proteolytic **18O** labeling and endoprotease-catalyzed 16O-to-**18O** exchange of truncated **peptide** substrates)

IT **9002-07-7, Trypsin**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(dissection of proteolytic **18O** labeling and endoprotease-catalyzed 16O-to-**18O** exchange of truncated **peptide** substrates)

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:658659 HCAPLUS
DOCUMENT NUMBER: 137:197868
TITLE: Phosphoprotein binding agents and methods of their use
INVENTOR(S): Goshe, Michael B.; Conrads, Thomas P.; Veenstra, Timothy D.; Panisko, Ellen A.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002119505	A1	20020829	US 2001-788286	20010216
WO 2002066988	A2	20020829	WO 2002-US4564	20020215

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE,

CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-788286 A 20010216

AB The invention provides reagents and methods for characterizing (i.e., identification and/or quantitation) the phosphorylation states of **proteins**. **Proteins** may be post-transcriptionally modified such that they contain phosphate groups at either some or all of their serine, threonine, tyrosine, histidine, and/or lysine amino acid residues. In many cases the extent to which a **protein** is phosphorylated dets. its bioactivity, i.e., its ability to effect cell functions such as differentiation, division, and metab. Hence, a powerful tool for diagnosing various diseases and for furthering the understanding of **protein-protein** interactions is provided. Two equal .beta.-casein samples were labeled with ethanedithiol (EDT) or EDT-2H4, resp., under .beta.-elimination conditions with NaOH. The labeled samples were quenched, desalted, denatured, reduced, biotinylated with iodoacetyl-PEO-biotin, and digested with **trypsin**. The labeled **peptides** were purified by affinity chromatog. using immobilized avidin and analyzed capillary reversed-phase liq. chromatog.-**mass spectrometry**.

IT **7782-39-0, 2H**, uses **14390-96-6, 15N**, uses **14762-74-4, 13C**, uses **14797-71-8, 18O**, uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(as label; phosphoprotein binding agents and methods of use)

IT **9002-07-7, Trypsin**
RL: CAT (Catalyst use); USES (Uses)
(phosphoprotein binding agents and methods of use)

L7 ANSWER 7 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:539911 HCAPLUS
DOCUMENT NUMBER: 137:90576
TITLE: Stable isotope, site-specific mass tagging for **protein** identification
INVENTOR(S): Chen, Xian
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002055989	A2	20020718	WO 2002-US538	20020111
WO 2002055989	A3	20021128		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2002146743 A1 20021010 US 2002-43965 20020111
PRIORITY APPLN. INFO.: US 2001-261716P P 20010112

AB Proteolytic **peptide** mass mapping as measured by **mass spectrometry** provides an important method for the identification of **proteins**, which are usually identified by matching the measured and calcd. m/z values of the proteolytic **peptides**. A unique identification is, however, heavily dependent upon the mass accuracy and sequence coverage of the fragment ions generated by **peptide** ionization. The present invention describes a method for increasing the specificity, accuracy and efficiency of the assignments of particular proteolytic **peptides** and consequent **protein** identification, by the incorporation of selected amino acid residue(s) enriched with stable isotope(s) into the **protein** sequence without the need for ultrahigh instrumental accuracy. Selected amino acids(s) are labeled. With **13C/15N/2H** and incorporated into **proteins** in a sequence-specific manner during cell culturing. Each of these labeled amino acids carries a defined mass change encoded in its monoisotopic distribution pattern. Through their characteristic patterns, the **peptides** with mass tag(s) can be readily distinguished from other **peptides** in mass spectra. The present method of identifying unique **proteins** can also be extended to **protein** complexes and will significantly increase data search specificity, efficiency and accuracy for **protein** identifications.

IT **7782-39-0, Hydrogen-2**, uses
14390-96-6, Nitrogen-15, uses
14762-74-4, Carbon-13, uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(stable isotope, site-specific mass tagging for **protein** identification)

IT **9001-92-7**, Protease **9002-07-7, Trypsin**
RL: CAT (Catalyst use); USES (Uses)
(stable isotope, site-specific mass tagging for **protein** identification)

L7 ANSWER 8 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:505014 HCAPLUS
DOCUMENT NUMBER: 137:59881
TITLE: Inverse labeling method for the rapid identification of marker/target **proteins**
INVENTOR(S): Wang, Yingqi Karen; Ma, Zhixiang; Quinn, Douglas Frederick; Fu, Emil W.
PATENT ASSIGNEE(S): Novartis A.-G., Switz.; Novartis-Erfindungen Verwaltungsgesellschaft m.b.H.
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002052271	A2	20020704	WO 2001-EP15228	20011221
WO 2002052271	A3	20021031		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LT, LU, LV, MA, MD, MK, MN, MX, NO, NZ, OM, PH, PL, PT, RO, RU, SE, SG, SI, SK, TJ, TM, TN, TR, TT, UA, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

US 2002090652	A1	20020711	US 2001-16627	20011210

PRIORITY APPLN. INFO.: US 2000-257559P P 20001222
US 2001-332965P P 20011119

AB A novel procedure for performing **protein** labeling for comparative proteomics termed inverse labeling is provided for the rapid identification of marker or target **proteins**. With this method, to evaluate **protein** expression of a disease or a drug treated sample in comparison with a control sample, two converse collaborative labeling expts. are performed in parallel. In one expt. the perturbed sample (by disease or by drug treatment) is isotopically heavy-labeled, whereas, the control is isotopically heavy-labeled in the second expt. When mixed and analyzed with its unlabeled or isotope light counterpart for differential comparison, a characteristic inverse labeling pattern is obsd. between the two parallel analyses for **proteins** that are differentially expressed to an appreciable level. In particularly useful embodiments, **protein** labeling is achieved through proteolytic **18O**-incorporation into **peptides** as a result of proteolysis performed in **18O**-water, metabolic incorporation of **15N** (or **13C** and **2H**) into **proteins**, and chem. tagging **proteins** with an isotope-coded tag reagent such as an isotope-coded affinity tag reagent.

IT **7782-39-0, Deuterium**, biological studies
14390-96-6, 15N, biological studies
14762-74-4, 13C, biological studies
14797-71-8, Oxygen-18, biological studies
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); RCT (Reactant); ANST (Analytical study); BIOL (Biological study); RACT (Reactant or reagent); USES (Uses)
(inverse labeling method for rapid identification of marker/target **proteins**)

IT **9002-07-7, Trypsin**
RL: CAT (Catalyst use); USES (Uses)
(inverse labeling method for rapid identification of marker/target **proteins**)

L7 ANSWER 9 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:466317 HCAPLUS
DOCUMENT NUMBER: 137:43912
TITLE: Acid-labile isotope-coded extractant (ALICE) and its use in quantitative **mass spectrometric** analysis of **protein** mixtures
INVENTOR(S): Qiu, Yongchang; Wang, Jack H.; Hewick, Rodney M.

PATENT ASSIGNEE(S): Genetics Institute, Inc., USA
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002048717	A2	20020620	WO 2001-US50745	20011022
WO 2002048717	A3	20030501		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002043385	A5	20020624	AU 2002-43385	20011022
US 2002164809	A1	20021107	US 2001-45170	20011022
EP 1330654	A2	20030730	EP 2001-989278	20011022

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRIORITY APPLN. INFO.: US 2000-242643P P 20001023
WO 2001-US50745 W 20011022

AB The invention concerns a method which provides novel compds., termed acid-labile isotope-coded extractants (ALICE), for quant. **mass spectrometric** anal. of **protein** mixts. The compds. contain a thiol-reactive group that is used to capture cysteine-contg. **peptides** from all **peptide** mixts., an acid-labile linker, and a non-biol. polymer. One of the two acid-labile linkers is isotopically labeled and therefore enables the direct quantitation of **peptides**/**proteins** through **mass spectrometric** anal. Because no functional **proteins** are required to capture **peptides**, a higher percentage of org. solvent can be used to solubilize the **peptides**, particularly hydrophobic **peptides**, through the binding, washing and eluting steps, thus permitting much better recovery of **peptides**. Moreover, since the **peptides** are covalently linked to the non-biol. polymer (ALICE), more stringent washing is allowed in order to completely remove non-specifically bound species. Finally, **peptides** captured by ALICE are readily eluted from the polymer support under mild acid condition with high yield and permit the direct down stream **mass spectrometric** anal. without any further sample manipulation. In combination with our novel dual column two dimensional liq. chromatog.- **mass spectrometry** (2D-LC-**MS**/**MS**) design, the ALICE procedure proves to a general approach for quant. **mass spectrometric** anal. of **protein** mixts. with better dynamic range and sensitivity.

IT **9002-07-7, Trypsin**
RL: NUU (Other use, unclassified); USES (Uses)

(acid-labile isotope-coded extractant (ALICE) and use in quant. **mass spectrometric** anal. of **protein** mixts.)

IT **7782-39-0, Deuterium,** properties
RL: PRP (Properties)
(acid-labile isotope-coded extractant (ALICE) and use in quant. **mass spectrometric** anal. of **protein** mixts.)

L7 ANSWER 10 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:450009 HCAPLUS
DOCUMENT NUMBER: 137:17454
TITLE: Isotope-coded ionization-enhancing reagents (ICIER) for high-throughput **protein** identification and quantitation using matrix-assisted laser desorption ionization **mass spectrometry**
INVENTOR(S): Qiu, Yongchang; Wang, Jack H.; Hewick, Rodney M.
PATENT ASSIGNEE(S): Genetics Institute, LLC, USA
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002046770	A2	20020613	WO 2001-US50744	20011022
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002041732	A5	20020618	AU 2002-41732	20011022
US 2003054570	A1	20030320	US 2001-44708	20011022

PRIORITY APPLN. INFO.: US 2000-242645P P 20001023
WO 2001-US50744 W 20011022

AB The invention concerns arginine-contg. cysteine-modifying compds. useful for MALDI-MS anal. of **proteins** are provided. These compds. termed isotope-coded ionization enhancement reagents (ICIER) can provide ionization enhancement in MALDI-MS, relative quantitation, and addnl. database searching constraints at the same time without any extra sample manipulation. More specifically, ICIER increase the ionization efficiency of cysteine-contg. **peptides** by attachment of a guanidino functional group. ICIER also increase the overall hydrophilicity of these **peptides** due the hydrophilic nature of ICIER and thus increase the percentage of recovery of these **peptides** during sample handling and processing such as in-gel digestion or liq. chromatog. Finally, a combination of both light and heavy ICIER provides an accurate way to obtain relative quantitation of

proteins by MALDI-MIS and addnl. database searching constraints (no. of cysteine residues in every single **peptide** peak) to increase the confidence of **protein** identification by **peptide** mass mapping.

IT **7782-39-0, Deuterium**, uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(isotope-coded ionization-enhancing reagents (ICIER) for high-throughput **protein** identification and quantitation using matrix-assisted laser desorption ionization **mass spectrometry**)

IT **9001-92-7**, Proteinase
RL: NUU (Other use, unclassified); USES (Uses)
(isotope-coded ionization-enhancing reagents (ICIER) for high-throughput **protein** identification and quantitation using matrix-assisted laser desorption ionization **mass spectrometry**)

L7 ANSWER 11 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:351546 HCAPLUS
DOCUMENT NUMBER: 137:274605
TITLE: Molecular dissection of membrane-transport **proteins: mass spectrometry** and sequence determination of the galactose-H+ symport **protein**, GalP, of Escherichia coli and quantitative assay of the incorporation of [ring-2-**13C**]histidine and 15NH3
AUTHOR(S): Venter, Henrietta; Ashcroft, Alison E.; Keen, Jeffrey N.; Henderson, Peter J. F.; Herbert, Richard B.
CORPORATE SOURCE: Astbury Centre for Structural Molecular Biology, School of Biochemistry and Molecular Biology, University of Leeds, Leeds, LS2 9JT, UK
SOURCE: Biochemical Journal (2002), 363(2), 243-252
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The mol. mass of the galactose-H+ symport **protein** GalP, as its histidine-tagged deriv. GalP(His)6, has been detd. by electrospray MS (ESI-MS) with an error of < 0.02%. One methionine residue, predicted to be present from the DNA sequence, was deduced to be absent. This is a significant advance on the estn. of the mol. masses of membrane-transport **proteins** by SDS/PAGE, where there is a consistent underestimation of the true mol. mass due to anomalous **electrophoretic** migration. Addn. of a size-exclusion chromatog. step after Ni2+-nitrilotriacetate affinity purifn. was essential to obtain GalP(His)6 suitable for ESI-MS. Controlled **trypsin**, **trypsin**+chymotrypsin and CNBr digestion of the **protein** yielded **peptide** fragments suitable for ESI-MS and tandem MS anal., and accurate mass detn. of the derived fragments resulted in identification of 82% of the GalP(His)6 **protein**. Tandem MS anal. of selected **peptides** then afforded 49% of the actual amino acid sequence of the **protein**; the absence of the N-terminal methionine was confirmed. Matrix-assisted laser-desorption ionization MS allowed identification of one **peptide** that was not

detected by ESI-MS. All the **protein/peptide** mass and sequence detns. were in accord with the predictions of amino acid sequence deduced from the DNA sequence of the galP gene. [Ring-2-**13C**]Histidine was incorporated into GalP(His)6 in vivo, and ESI-MS anal. enabled the measurement of a high (80%) and specific incorporation of label into the histidine residues in the **protein**. MS could also be used to confirm the labeling of the **protein** by 15NH3 (93% enrichment) and [19F] tryptophan (83% enrichment). Such MS measurements will serve in the future anal. of the structures of membrane-transport **proteins** by NMR, and of their topol. by indirect techniques.

IT **9002-07-7, Trypsin**
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(GalP digestion by; **mass spectrometry** and sequence detn. of galactose-H+ symport **protein** GalP of E. coli and quant. assay of incorporation of [ring-2-**13C**]histidine and 15NH3)

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 12 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:172146 HCAPLUS
DOCUMENT NUMBER: 136:213181
TITLE: **Mass spectrometric** analysis of **biopolymers**
INVENTOR(S): Paech, Christian; Paech, Sigrid; Estell, David A.; Ganshaw, Grant C.
PATENT ASSIGNEE(S): Genencor International, Inc., USA
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002018644	A2	20020307	WO 2001-US25884	20010817
WO 2002018644	A3	20030116		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001085063	A5	20020313	AU 2001-85063	20010817
US 2002072064	A1	20020613	US 2001-932279	20010817
US 2002123055	A1	20020905	US 2001-932369	20010817
EP 1311707	A2	20030521	EP 2001-964178	20010817
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC,				

PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
PRIORITY APPLN. INFO.: US 2000-228198P P 20000825
WO 2001-US25884 W 20010817

AB The present invention makes use of unique tags of a specific **biopolymer** that can be exploited for detg. the concn. the **biopolymer** in crude solns. In preferred embodiments the **biopolymer** is either a **protein** or a polynucleotide. Particularly, the invention provides a method for the detn. and quantitation of **biomols.** in crude mixts. by way of a **sepn.** technique in combination with mass spectroscopy. In one general embodiment, a target **biomol.** is selected for anal. and an analog thereof is generated. Peak area integration of the **peptide** pairs provides a direct measure for the amt. of target **protein** in the crude soln. A **15N**-labeled subtilisin mutant expressed by Bacillus subtilis and wildtype B. lentus subtilisin were studied by **peptide** maping and **mass spectrometry.**

IT **7782-39-0, Deuterium,** uses **14390-96-6, Nitrogen-15,** uses **14762-74-4, Carbon-13,** uses **14797-71-8, Oxygen-18,** uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(as label; **mass spectrometric** anal. of **biopolymers**)

IT **9001-92-7, Proteolytic enzyme**
9002-07-7, Trypsin
RL: CAT (Catalyst use); USES (Uses)
(**mass spectrometric** anal. of **biopolymers**)

L7 ANSWER 13 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:153750 HCAPLUS
DOCUMENT NUMBER: 136:365964
TITLE: Proteolytic **18O** labeling for comparative proteomics: Evaluation of endoprotease glu-C as the catalytic agent
AUTHOR(S): Reynolds, Kristy J.; Yao, Xudong; Fenselau, Catherine
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20742, USA
SOURCE: Journal of Proteome Research (2002), 1(1), 27-33
CODEN: JPROBS; ISSN: 1535-3893
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recently, proteolytic **18O** labeling has been demonstrated as a promising strategy for comparative proteomic studies (Yao, X.; Freas, A.; Ramirez, J.; Demirev, P. A.; Fenselau, C. Anal. Chem. 2001, 73, 2836-42). In this approach, **protein** mixts. are digested in parallel in H216O and H218O and the ratios of isotopically distinct **peptide** products are measured by **mass spectrometry.** In the initial report from this lab., **trypsin** was shown to catalyze incorporation of two **18O** atoms into the carboxyl terminus of each new **peptide** formed by cleavage of the adenovirus proteome. In the present study, a second enzyme, endoprotease Glu-C, is evaluated

as an agent for cleavage and labeling. Proteolytic **18O** labeling by Glu-C is shown to occur readily with phosphorylated and glycosylated **proteins** and with cysteine-alkylated and disulfide-linked **proteins**. A sequential double-labeling strategy is used to characterize N-linked glycopeptides. Labeled and unlabeled **peptide** pairs are found to coelute chromatog., and measurements of isotope ratios by nanospray and capillary LC-MS are found to be accurate and precise.

IT **14797-71-8**, **Oxygen-18**, analysis
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(proteolytic **18O** labeling for comparative proteomics)

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 14 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:924099 HCAPLUS
DOCUMENT NUMBER: 136:50669
TITLE: Selective labeling and isolation of phosphopeptides and applications to proteome analysis
INVENTOR(S): Aebersold, Ruedi; Zhou, Hullin
PATENT ASSIGNEE(S): University of Washington, USA
SOURCE: PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001096869	A1	20011220	WO 2001-US18988	20010612
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1295123	A1	20030326	EP 2001-944486	20010612
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2002049307	A1	20020425	US 2001-880713	20011018

PRIORITY APPLN. INFO.: US 2000-210972P P 20000612
WO 2001-US18988 W 20010612

AB A method for selective labeling of phosphate groups in natural and synthetic oligomers and polymers in the presence of chem. related groups such as carboxylic acid groups. The method is specifically applicable to biol. oligomers and polymers, including phosphopeptides, phosphoproteins and phospholipids. In a specific embodiment, selective labeling of phosphate groups in **proteins** and **peptides**, for example, facilitates **sepn.**, isolation and detection of phosphoproteins and

phosphopeptides in complex mixts. of **proteins**. Selective labeling can be employed to selectively introduce phosphate labels at phosphate groups in an oligomer or polymer, e.g., in a **peptide** or **protein**. Dection of the presence of the label, is used to detect the presence of the phosphate group in the oligomer or polymer. The method is useful for the detection of phosphoproteins or phosphopeptides. The phosphate label can be a colorimetric label, a radiolabel, a fluorescent or phosphorescent label, an affinity label or a linker group carrying a reactive group (or latent reactive group) that allows selective attachment of the oligomer of polymer (**protein** or **peptide**) to a phosphate label, to an affinity label or to a solid support. The method can be combined with well-known methods of **mass spectrometry** to detect and identify phosphopeptides and phosphoproteins.

IT **7782-39-0, Deuterium**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(selective labeling and isolation of phosphopeptides and applications to proteome anal.)

IT **9002-07-7, Trypsin**
RL: CAT (Catalyst use); USES (Uses)
(selective labeling and isolation of phosphopeptides and applications to proteome anal.)

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 15 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:904715 HCAPLUS
DOCUMENT NUMBER: 136:17697
TITLE: Labeling of proteomic samples during proteolysis for quantitation and sample multiplexing
INVENTOR(S): Figeys, Joseph Michel Daniel; Mann, Matthias; Stewart, Ian I.
PATENT ASSIGNEE(S): MDS Proteomics, Inc., Can.
SOURCE: PCT Int. Appl., 68 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001094935	A2	20011213	WO 2001-IB1328	20010608
WO 2001094935	A3	20021121		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1290450	A2	20030312	EP 2001-949829	20010608

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

US 2002076817 A1 20020620 US 2001-878750 20010611

PRIORITY APPLN. INFO.: US 2000-210496P P 20000609
US 2001-293664P P 20010525
WO 2001-IB1328 W 20010608

AB This invention relates to methods useful in the labeling of multiple **polypeptide** samples and subsequent anal. of these samples by **mass spectrometry**, particularly in the high throughput proteomic setting.

IT **14797-71-8, 18O**, analysis
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(labeling of proteomic samples during proteolysis for quantitation and sample multiplexing)

IT **9002-07-7, Trypsin**
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(labeling of proteomic samples during proteolysis for quantitation and sample multiplexing)

L7 ANSWER 16 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:244359 HCAPLUS
DOCUMENT NUMBER: 135:2188
TITLE: Characterization of differently processed forms of enolase 2 from Saccharomyces cerevisiae by two-dimensional gel **electrophoresis** and **mass spectrometry**
AUTHOR(S): Larsen, Martin R.; Larsen, Peter Mose; Fey, Stephen J.; Roepstorff, Peter
CORPORATE SOURCE: Department of Biochemistry Molecular Biology Center for Proteome Analysis, University of Southern Denmark, Odense University, Odense, DK-5230, Den.
SOURCE: Electrophoresis (2001), 22(3), 566-575
CODEN: ELCTDN; ISSN: 0173-0835
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two-dimensional gel **electrophoresis**, bioinformatics, and **mass spectrometry** are key anal. tools in proteome anal. The further characterization of post-translational modifications in gel-**sepd. proteins** relies fully on data obtained by **mass spectrometric** anal. In this study, stress-induced changes in **protein** expression in Saccharomyces cerevisiae were investigated. A total of eleven spots on a silver-stained two-dimensional (2-D) gel were identified by matrix-assisted laser desorption/ionization (MALDI) **peptide** mass mapping to represent C- and/or N-terminal processed forms of enolase 2. The processing sites were detd. by MALDI **peptide** mass mapping using a variety of **proteolytic enzymes**, by optimizing the sample prepn. procedure and by specific labeling of all C-termini derived from in-gel digestion using a buffer contg. 16O:**18O** (1:1). Out of eleven processed forms of enolase 2, six were fully characterized and the approx. processing sites identified for the remaining five.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE

IN THE RE FORMAT

L7 ANSWER 17 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:170837 HCAPLUS
DOCUMENT NUMBER: 134:350210
TITLE: Quantitative analysis of bacterial and mammalian proteomes using a combination of cysteine affinity tags and **15N**-metabolic labeling
AUTHOR(S): Conrads, Thomas P.; Alving, Kim; Veenstra, Timothy D.; Belov, Mikhail E.; Anderson, Gordon A.; Anderson, David J.; Lipton, Mary S.; Pasa-Tolic, Lijliana; Udseth, Harold R.; Chrisler, William B.; Thrall, Brian D.; Smith, Richard D.
CORPORATE SOURCE: Environmental and Molecular Sciences Laboratory and Molecular Biosciences Department, Pacific Northwest National Laboratory, Richland, WA, 99352, USA
SOURCE: Analytical Chemistry (2001), 73(9), 2132-2139
CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We describe the combined use of **15N**-metabolic labeling and a cysteine-reactive biotin affinity tag to isolate and quantitate cysteine-contg. **polypeptides** (Cys-**polypeptides**) from Deinococcus radiodurans as well as from mouse B16 melanoma cells. D. radiodurans were cultured in both natural isotopic abundance and **15N**-enriched media. Equal nos. of cells from both cultures were combined and the sol. **proteins** extd. This mixt. of isotopically distinct **proteins** was derivatized using a com. available cysteine-reactive reagent that contains a biotin group. Following **trypsin** digestion, the resulting modified **peptides** were isolated using immobilized avidin. The mixt. was analyzed by capillary reversed-phase liq. chromatog. (LC) online with ion trap **mass spectrometry** (**MS**) as well as Fourier transform ion cyclotron resonance (FTICR) **MS**. The resulting spectra contain numerous pairs of Cys-**polypeptides** whose mass difference corresponds to the no. of nitrogen atoms present in each of the **peptides**. Designation of Cys-**polypeptide** pairs is also facilitated by the distinctive isotopic distribution of the **15N**-labeled **peptides** vs. their 14N-labeled counterparts. Studies with mouse B16 cells maintained in culture allowed the observation of hundreds of isotopically distinct pairs of **peptides** by LC-FTICR anal. The ratios of the areas of the pairs of isotopically distinct **peptides** showed the expected 1:1 labeling of the 14N and **15N** versions of each **peptide**. An addnl. benefit from the present strategy is that the **15N**-labeled **peptides** do not display significant isotope-dependent chromatog. shifts from their 14N-labeled counterparts, therefore improving the precision for quantitating **peptide** abundances. The methodol. presented offers an alternate, cost-effective strategy for conducting global, quant. proteomic measurements.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE

FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 18 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:790729 HCAPLUS
DOCUMENT NUMBER: 133:331760
TITLE: Method for the comparative quantitative analysis of **proteins** and other biological material by isotopic labeling and mass spectroscopy
INVENTOR(S): Chait, Brian T.; Cowburn, David; Oda, Yoshi
PATENT ASSIGNEE(S): The Rockefeller University, USA
SOURCE: PCT Int. Appl., 55 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000067017	A1	20001109	WO 2000-US12026	20000503
W: CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6391649	B1	20020521	US 1999-304799	19990504
US 2003077840	A1	20030424	US 2001-949510	20010906

PRIORITY APPLN. INFO.: US 1999-304799 A 19990504

AB The present invention is a method for accurately comparing the levels of cellular components, such as **proteins**, present in samples which differ in some respect from each other using mass spectroscopy and isotopic labeling. A first sample of biol. matter, such as cells, is cultured in a first medium and a second sample of the same biol. matter is cultured in a second medium, wherein at least one isotope in the second medium has a different abundance than the abundance of the same isotope in the first medium. One of the samples is modulated, such as by treatment with a bacteria, a virus, a drug, hormone, a chem. or an environmental stimulus. The samples are combined and at least one **protein** is removed. The removed **protein** is subjected to mass spectroscopy to develop a mass spectrum. A ratio is computed between the peak intensities of at least one closely spaced pair of peaks to det. the relative abundance of the **protein** in each sample. The **protein** is identified by the mass spectrum or through other techniques known in the art. Modifications to the **proteins**, such as the phosphorylation of the **protein**, and the site of the modification may also be detd. through the process of the present invention. The method is applicable to the components of any type of biol. matter which are ionizable and may therefore be analyzed by mass spectroscopy.

IT **7782-39-0, Hydrogen 2**, uses
14390-96-6, Nitrogen-15, uses
14762-74-4, Carbon-13, uses
14797-71-8, Oxygen-18, uses
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(method for comparative quant. anal. of **proteins** and other biol. material by isotopic labeling and mass spectroscopy)

IT **9001-92-7, Proteolytic enzyme**
9002-07-7, Trypsin
RL: CAT (Catalyst use); USES (Uses)
(method for comparative quant. anal. of **proteins** and other biol. material by isotopic labeling and mass spectroscopy)
REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 19 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:699777 HCAPLUS
DOCUMENT NUMBER: 134:52347
TITLE: Characterization of Benzoquinone-**Peptide** Adducts by Electrospray **Mass Spectrometry**
AUTHOR(S): Mason, Daniel E.; Liebler, Daniel C.
CORPORATE SOURCE: Department of Pharmacology and Toxicology and Southwest Environmental Health Sciences Center College of Pharmacy, University of Arizona, Tucson, AZ, 85721-0207, USA
SOURCE: Chemical Research in Toxicology (2000), 13(10), 976-982
CODEN: CRTOEC; ISSN: 0893-228X
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Benzoquinone adducts were prepd. with model **peptides** to identify characteristic features of adduct fragmentation in tandem **mass spectrometry** (**MS**) expts. Model **peptides** contained cysteine and had a mol. mass of less than 2 kDa to facilitate **peptide** fragmentation in tandem MS analyses. **Peptides** were adducted with an excess of benzoquinone, and the adducts were analyzed by LC/MS. Adducts were identified by addn. of 108 Da to the monoisotopic mass of the **peptide**, except in the case of oxytocin, which formed a bis adduct with addn. of 216 Da. Tandem MS expts. were performed on the [M + **2H**]2+ ions and/or the [M + H]+ ions. Sequence information obtained from modified **peptides** was comparable to that of their unmodified counterparts. A unique ion pair **sepd**. by 141 or 142 Da corresponding to .beta.-elimination of benzoquinol-S or benzoquinol-SH from a bn or yn series ion indicated attachment at the sulfur of the cysteine residue. An alternate ion pair of 211 Da corresponded to fragmentation at the **peptide** bond on either side of the adducted cysteine. Enzymic digestion of BSA and a 2560 Da frog **peptide** with **trypsin** yielded tryptic **peptides**, which were treated with benzoquinone. In addn. to ion pairs of 142 and 211 Da, singly and doubly charged tryptic **peptide** adducts showed a neutral loss of 142 Da from the precursor. Either one or both ion pairs were present in more than half of all the **peptides** that were examd. The neutral loss of 142 Da was present in all singly charged tryptic **peptide** adducts and in 11 out of 14 doubly charged tryptic **peptide** adducts. The data indicate that reliable detection of benzoquinone-cysteinyl **peptide** adducts requires monitoring of multiple spectral characteristics.
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 20 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:633407 HCAPLUS
DOCUMENT NUMBER: 133:319239
TITLE: Two-dimensional **electrophoretic** /chromatographic **separations** combined with electrospray ionization FTICR **mass spectrometry** for high throughput proteome analysis
AUTHOR(S): Gao, Hongying; Shen, Yufeng; Veenstra, Timothy D.; Harkewicz, Richard; Anderson, Gordon A.; Bruce, James E.; Pasa-Tolic, Ljiljana; Smith, Richard D.
CORPORATE SOURCE: Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, 99352, USA
SOURCE: Journal of Microcolumn Separations (2000), 12(7), 383-390
CODEN: JMSEEJ; ISSN: 1040-7685
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A two-dimensional **sepn.** strategy combined with electrospray ionization-Fourier transform ion cyclotron resonance **mass spectrometry** (ESI-FTICR) is being developed for high throughput proteomic analyses. Capillary isoelec. focusing (CIEF) coupled online with a robotic fraction collector is used to **sep.** and collect microliter fractions of sol. Saccharomyces cerevisiae (yeast) **proteins** eluting from the capillary into a microtiter plate. Following tryptic digestion of each fraction, the resultant **peptides** are **sepd.** using capillary high performance liq. chromatog. (**HPLC**) and analyzed by online ESI-FTICR. **Protein** identification is based upon the use of the exptl. measured **peptide** masses as accurate mass tags, augmented by conventional MS/MS methods as necessary, to identify **proteins** predicted from the yeast genome sequence. This new **sepn.** strategy is being evaluated using **proteins** extd. from yeast grown in natural isotopic abundance and **15N**-enriched media. Two isotopically distinct versions of each **peptide** are thus obsd. in the ESI-FTICR spectra. The mass differences between the two versions are used to det. the no. of nitrogen atoms in the **peptide**, and provide an addnl. constraint that aids **protein** identification. More importantly, the use of this stable-isotope labeling strategy enables the generation of "comparative displays" of the precise relative **protein** abundances. This two-dimensional **sepn.** strategy combined with ESI-FTICR anal. is expected to be highly amenable to automation.

IT **9002-07-7, Trypsin**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(two-dimensional **electrophoretic**/chromatog. **sepns.** combined with electrospray ionization FTICR **mass spectrometry** for high throughput proteome anal.)

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE

IN THE RE FORMAT

L7 ANSWER 21 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:457236 HCAPLUS
DOCUMENT NUMBER: 133:55646
TITLE: Methods for quantifying heavy hydrogen levels at specific **peptide** amide linkages in **proteins** and **peptides** by fragmentation and mass determination
INVENTOR(S): Smith, David L.; Deng, Yuzhong; Pan, Hai
PATENT ASSIGNEE(S): Board of Regents of the University of Nebraska, USA
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000039326	A1	20000706	WO 1999-US30574	19991221

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1998-113772P P 19981223

AB A method of detg. the amt. of **deuterium** at **peptide** amide linkages in a **polypeptide**, at the resoln. of a single amino acid, is disclosed. A **polypeptide** is fragmented below a scrambling threshold into a plurality of **peptide** subfragments. The amt. of **deuterium** assocd. with each of the **peptide** subfragments is quantified by detg. its mass. The amt. of **deuterium** in each of the **peptide** subfragments is correlated with the amino acid sequence of the **polypeptide** to det. the amt. of **deuterium** at **peptide** amide linkages.

IT **9001-92-7**, Protease
RL: CAT (Catalyst use); NUU (Other use, unclassified); USES (Uses)
(methods for quantifying heavy hydrogen levels at specific **peptide** amide linkages in **proteins** and **peptides** by fragmentation and mass detn.)

IT **7782-39-0**, **Deuterium**, processes
RL: PEP (Physical, engineering or chemical process); PROC (Process)
(methods for quantifying heavy hydrogen levels at specific **peptide** amide linkages in **proteins** and **peptides** by fragmentation and mass detn.)

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 22 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:145059 HCAPLUS

DOCUMENT NUMBER: 132:191408
TITLE: Rapid quantitative analysis of **proteins** or **protein** function in complex mixtures using affinity labeling reagents and **mass spectrometry**
INVENTOR(S): Aebersold, Rudolf Hans; Gelb, Michael H.; Gygi, Steven P.; Scott, C. Ronald; Turecek, Frantisek; Gerber, Scott A.; Rist, Beate
PATENT ASSIGNEE(S): University of Washington, USA
SOURCE: PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000011208	A1	20000302	WO 1999-US19415	19990825
W: AU, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9956913	A1	20000314	AU 1999-56913	19990825
AU 755334	B2	20021212		
EP 1105517	A1	20010613	EP 1999-943915	19990825
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002523058	T2	20020730	JP 2000-566460	19990825
JP 3345401	B2	20021118		
JP 2003107066	A2	20030409	JP 2002-208687	19990825
EP 1329513	A1	20030723	EP 2003-75828	19990825
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
US 2002076739	A1	20020620	US 2001-839884	20010420
US 2003087322	A9	20030508		

PRIORITY APPLN. INFO.:

US 1998-97788P	P	19980825
US 1998-99113P	P	19980903
EP 1999-943915	A3	19990825
JP 2000-566460	A3	19990825
US 1999-383062	A3	19990825
WO 1999-US19415	W	19990825

OTHER SOURCE(S): MARPAT 132:191408

AB Anal. reagents and **mass spectrometry**-based methods using these reagents for the rapid, and quant. anal. of **proteins** or **protein** function in mixts. of **proteins** are disclosed. The methods employ affinity labeled **protein** reactive reagents having three portions: an affinity label (A) covalently linked to a **protein** reactive group (PRG) through a linker group (L). The linker may be differentially isotopically labeled, e.g., by substitution of one or more atoms in the linker with a stable isotope thereof. These reagents allow for the selective isolation of **peptide** fragments or the products of reaction with a given **protein** (e.g., products of enzymic reaction) from complex mixts. The isolated **peptide** fragments or reaction products are characteristic of the presence of a **protein** or the presence of a **protein** function in those mixts. Isolated **peptides** or reaction products are characterized by **mass**

spectrometric (**MS**) techniques. The reagents also provide for differential isotopic labeling of the isolated **peptides** or reaction products which facilitates quant. detn. by **mass spectrometry** of the relative amt. of **proteins** in different samples. The methods of this invention can be used for qual. and quant. anal. of global **protein** expression profiles in cells and tissues, to screen for and identify **proteins** whose expression level in cells, tissue or biol. fluids is affected by a stimulus or by a change in condition or cell state of the cell, tissue or organism from which the sample originated. A conjugate of N-methylglycylbiotinamide acid and the Michael addn. product of 4,7,10-trioxa-1,13-tridecanediamine and p-acrylamidophenyl-.beta.-D-galactopyranoside was prepd. for detecting .beta.-D-galactosidase deficiency and GM1-gangliosidosis.

IT **9001-92-7, Proteolytic enzyme**
RL: ARU (Analytical role, unclassified); CAT (Catalyst use); ANST (Analytical study); USES (Uses)
(rapid quant. anal. of **proteins** or **protein** function in complex mixts. using affinity labeling reagents and **mass spectrometry**)

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 23 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:713419 HCAPLUS
DOCUMENT NUMBER: 130:78415
TITLE: Modification of Cysteine Residues by Alkylation. A Tool in **Peptide** Mapping and **Protein** Identification
AUTHOR(S): Sechi, Salvatore; Chait, Brian T.
CORPORATE SOURCE: The Rockefeller University, New York, NY, 10021, USA
SOURCE: Analytical Chemistry (1998), 70(24), 5150-5158
CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Although **mass spectrometric peptide** mapping has become an established technique for the rapid identification of **proteins** isolated by PAGE, the results of the identification procedure can sometimes be ambiguous. Such ambiguities become increasingly prevalent for **proteins** isolated as mixts. or when only very small amts. of the **proteins** are isolated. The quality of the identification procedure can be improved by increasing the no. of **peptides** that are extd. from the gel. Here we show that cysteine alkylation is required to ensure maximal coverage in matrix-assisted laser desorption/ionization time-of-flight **mass spectrometry** (MALDI-TOF **MS**) **peptide** mapping of **proteins** isolated by PAGE. In the described procedure, alkylation was performed prior to **electrophoresis** to avoid the adventitious formation of acrylamide adducts during **electrophoresis**. In this way, homogeneous alkylation was obtained with three different alkylating reagents (4-vinylpyridine, iodoacetamide, acrylamide). Cysteine alkylation was also used as a tool for the identification of cysteine-contg. **peptides**.

Using a 1:1 mixt. of unlabeled acrylamide and **deuterium** -labeled acrylamide ([2,3,3'-D3]acrylamide), the **proteins** of interest were alkylated prior to **electrophoretic sepn.** **Peptide** mixts. produced by **trypsin** digestion of the resulting **protein** bands were analyzed by MALDI-TOF MS, and the cysteine content of the **peptides** was inferred from the isotopic distributions. The cysteine content information was readily obtained and used to improve the **protein** identification process.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 24 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:661168 HCAPLUS
DOCUMENT NUMBER: 130:63181
TITLE: **Peptide** sequencing by ESI-MS-MS
AUTHOR(S): Bordoli, R. S.; Kapp, E.; Langridge, J. I.
CORPORATE SOURCE: Micromass UK Ltd., Wythenshawe/Manchester, M23 9LZ, UK
SOURCE: Speciality Chemicals (1998), 18(7), 304-305
CODEN: SPCHEY; ISSN: 0262-2262
PUBLISHER: DMG Business Media Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Using **18O** labeling it has been shown that partial and complete de novo sequences maybe detd. from MS-MS spectra by software interpretation. In this expt. Golgi membrane **proteins** were purified by TX114 detergent extn. and **electrophoresis**; reduced with dithiothreitol and alkylated with iodoacetamide; and in-gel digested in buffer contg. 50 vol./vol.% **18O** water with **trypsin**. Q-Time-Of-Flight **Mass Spectrometry** hybrid tandem **mass spectrometer** sequence detn.

IT **9002-07-7, Trypsin**
RL: RCT (Reactant); RACT (Reactant or reagent)
(**peptide** sequencing by ESI-MS-MS and software interpretation)

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 25 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:707364 HCAPLUS
DOCUMENT NUMBER: 128:72570
TITLE: Quantitative analysis of exogenous **peptides** in plasma using immobilized enzyme cleavage and gas chromatography-**mass spectrometry** with negative ion chemical ionization
AUTHOR(S): Marquez, Cristina D.; Lee, Mu-Lan; Weintraub, Susan T.; Smith, Philip C.
CORPORATE SOURCE: College of Pharmacy, University of Texas at Austin, Austin, TX, 78712, USA
SOURCE: Journal of Chromatography, B: Biomedical Sciences and Applications (1997), 700(1 + 2), 9-21
CODEN: JCBBEP; ISSN: 0378-4347

PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A method is presented for the anal. of **peptides** in plasma at picomole to femtomole levels. **Peptides** are isolated from plasma by solid-phase extn., the **peptide** of interest is purified by reversed-phase high-performance liq. chromatog. (**HPLC**) and selectively digested using immobilized **trypsin** or chymotrypsin to yield specific di- or tripeptides. These di- and tripeptides are esterified using heptafluorobutyric anhydride, alkylated with pentafluorobenzyl bromide, then quantified by gas chromatog.-**mass spectrometry** with neg. ion chem. ionization. This method has been evaluated for a model synthetic heptapeptide, using a **deuterium** labeled analog as an internal std. The half-life of the heptapeptide in human plasma was found to be 2 min. Extn. efficiencies of a tritiated **peptide** of similar size to the heptapeptide, [3H]DSLET, from plasma using either C18 or strong cation-exchange columns were 85.+-.3 and 70.+-.2%, resp. Quantitation of fragments from the heptapeptide indicated that the anal. was linear from 1-50 ng of the heptapeptide per mL of plasma. This method was subsequently employed for pharmacokinetic studies of the biol. active **peptide** Met-enkephalin-Arg-Gly-Leu, where linearity was obtained from 50 to 1000 ng/mL in rat plasma. This method demonstrated negligible side reaction byproducts due to autolysis, and has potential for extensive use given the wide availability of gas chromatog.-**mass spectrometry**

IT **9002-07-7, Trypsin**
RL: ARG (Analytical reagent use); BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(anal. of **peptides** in plasma using immobilized enzyme cleavage and gas chromatog.-**mass spectrometry**
)

L7 ANSWER 26 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:319903 HCAPLUS
DOCUMENT NUMBER: 122:161323
TITLE: Macrocyclic **Peptide** Inhibitors of Serine Proteases. Convergent Total Synthesis of Cyclotheonamides A and B via a Late-Stage Primary Amine Intermediate. Study of Thrombin Inhibition under Diverse Conditions
AUTHOR(S): Maryanoff, Bruce E.; Greco, Michael N.; Zhang, Han-Cheng; Andrade-Gordon, Patricia; Kauffman, Jack A.; Nicolaou, K. C.; Liu, Aijun; Brungs, Peter H.
CORPORATE SOURCE: R. W. Johnson Pharmaceutical Research Institute, Spring House, PA, 19477, USA
SOURCE: Journal of the American Chemical Society (1995), 117(4), 1225-39
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
GI

* STRUCTURE DIAGRAM TOO LARGE FOR DISPLAY - AVAILABLE VIA OFFLINE PRINT *

AB Cyclotheonamide A (I; R = CHO) (II), a cyclic pentapeptide isolated from the marine sponge Theonella sp., is an inhibitor of serine proteases such as .alpha.-thrombin and **trypsin**. The total synthesis of II by a convergent [3 + 2] fragment-condensation route is described in detail. The requisite protected amino acid starting materials were processed and converted into two segments, III (TBS = Me3CSiMe2, PhtN = phthalimido) and IV (Fmoc = 9-fluorenylmethoxycarbonyl, Ts = tosyl), which were coupled with BOP reagent in 75% yield to give a pentapeptide intermediate. After selective removal of the terminal protecting groups, the crit. macrocyclization was effected with BOP-Cl in 65% yield under high-diln. conditions to provide V in 25% overall yield. Macrocycle V was then processed in four steps to II, which was isolated and purified by **HPLC** (trifluoroacetate salt). Synthetic II was identical to the natural product by 500 MHz 1H NMR, 100-MHz **13C** NMR, **HPLC**, TLC, fast-atom-bombardment **mass spectrometry**, optical rotation, and bioassay. The **13C** NMR spectrum of II in D2O shows virtually exclusive population by the hydrated form of the .alpha.-keto amide (gem-diol structure). Cyclotheonamide B (I; R = Ac) was also prepd. through an analogous transformation. This chem. protocol offers a useful vehicle for the systematic prepn. of cyclotheonamide analogs, and because of a the late-stage primary amine intermediate, analogs with a modified N-acyl or N-alkyl substituent should be conveniently accessible. This seems important for satisfying the hydrophobic S3 binding pocket of thrombin which is vacant for the CtA-thrombin complex but effectively utilized by the std. D-Phe-Pro-Arg tripeptide inhibitors. Other chem. highlights of the synthesis include (1) homologation of protected arginal via a cyanohydrin to obtain the homoarginine subunit, (2) use throughout of a monoprotected guanidine, and (3) macrocyclic lactam formation with an unprotected hydroxyl substituent. The characteristics of II as a thrombin inhibitor were also examd. Either competitive, Michaelis-Menten kinetics or slow, tight-binding kinetics were obsd., depending on the substrate, the thrombin concn., and the order of addn. of components. Given sufficient time for equilibration of II and thrombin, slow-binding inhibition is generally displayed.

L7 ANSWER 27 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1992:546541 HCAPLUS
DOCUMENT NUMBER: 117:146541
TITLE: A method for determination of N-glycosylation sites in glycoproteins by collision-induced dissociation analysis in fast atom bombardment **mass spectrometry**: identification of the positions of carbohydrate-linked asparagine in recombinant .alpha.-amylase by treatment with **peptide**-N-glycosidase F in **oxygen-18**-labeled water
AUTHOR(S): Gonzalez, Javier; Takao, Toshifumi; Hori,

Hideaki; Besada, Vladimir; Rodriguez, Rolando; Padron, Gabriel; Shimonishi, Yasutsugu
CORPORATE SOURCE: Inst. Protein Res., Osaka Univ., Suita, 565, Japan
SOURCE: Analytical Biochemistry (1992), 205(1), 151-8
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Previously, a combined use of fast atom bombardment (FAB) **mass spectrometry** and **peptide** N-glycosidase F, an enzyme that cleaves the .beta.-aspartylglycosylamine linkage of Asn-linked carbohydrates, was successfully applied to identification of N-glycosylation sites in a glycoprotein with the known or DNA-derived sequence (S. A. Carr; G. D. Roberts, 1986). Here, the method is extended for easier identification of N-glycosylation sites in a glycoprotein even with unknown sequence. The glycoprotein is digested with **peptide** -N-glycosidase F in buffer contg. 40 at. % H218O, to yield a deglycosylated **protein** whose carbohydrate-linked Asn residues are converted to Asp partly labeled with **18O** at their .beta.-carboxyl group during this digestion. The deglycosylated **protein** is further digested with **proteolytic enzymes** in an appropriate buffer prepd. with normal water, and then **peptides** are **sepd.** on a reversed-phase column by **HPLC**. **Peptides** in which carbohydrate-linked Asn has been converted to Asp show a pair of signals ([M + 1]+ and [M + 3]+) in FAB mass spectra due to the partial incorporation of **18O** into the .beta.-carboxyl groups of Asp residues, while the other **peptides** show normal isotopic ion distributions. Thus, both formally N-glycosylated **peptides** and, using collision-induced dissocn. anal., N-glycosylation sites can be identified. The application of the present method to the detn. of N-glycosylation sites in a recombinant glycoprotein, Bacillus licheniformis .alpha.-amylase, is described.

L7 ANSWER 28 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:545392 HCAPLUS
DOCUMENT NUMBER: 113:145392
TITLE: Optimization of immobilized enzyme hydrolysis combined with high-performance **liquid chromatography**/thermospray **mass spectrometry** for the determination of neuropeptides
AUTHOR(S): Voyksner, Robert D.; Chen, David C.; Swaisgood, Harold E.
CORPORATE SOURCE: Research Triangle Inst., Research Triangle Park, NC, 27709, USA
SOURCE: Analytical Biochemistry (1990), 188(1), 72-81
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Peptidases, including chymotrypsin, thermolysin, **trypsin**, V8 protease, and carboxypeptidases A, B, and Y, were immobilized for use in conjunction with **HPLC**/thermospray **mass spectrometry** for the anal. of neuropeptides. The optimal operating conditions for each immobilized enzyme bioreactor were detd. Optical hydrolysis usually occurred at the highest percentage

of aq. soln. in the mobile phase at pH 7-8 and 40-50.degree.. Often post-**HPLC** column addn. of aq. solns. before the bioreactor could improve activity and thermospray sensitivity without changing the **HPLC sepn.** Enzymic hydrolysis requirements were compatible under conditions for **HPLC sepn.** and thermospray **mass spectrometry** detection of the selected neuropeptides. Synthetic .alpha.-, .beta.-, and .gamma.-endorphins were the primary neuropeptides used to evaluate online immobilized enzyme bioreactor/**mass spectrometry**. **HPLC** followed by peptidase hydrolysis produced characteristic hydrolysis products for confirming the **peptides'** identity using thermospray **mass spectrometry** detection. Furthermore, the **peptide** formed from enzymic hydrolysis resulted in a **mass spectrometry** ion current 10-40 times higher than that of the [M + **2H**]2+ ion for unhydrolyzed .beta.-endorphin. The increased sensitivity achieved for detecting the hydrolysis products permits detection and quantitation of synthetic **peptides** down to 800 fmol.

IT **9002-07-7, Trypsin**
RL: BIOL (Biological study)
(immobilized, neuropeptides detn. by **HPLC**/thermospray **mass spectrometry** and)

L7 ANSWER 29 OF 29 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1988:127939 HCAPLUS
DOCUMENT NUMBER: 108:127939
TITLE: C-Terminal **peptide** identification by fast atom bombardment **mass spectrometry**
AUTHOR(S): Rose, Keith; Savoy, Luc Alain; Simona, Marco G.; Offord, Robin E.; Wingfield, Paul
CORPORATE SOURCE: Dep. Biochim. Med., Cent. Med. Univ., Geneva, CH-1211, Switz.
SOURCE: Biochemical Journal (1988), 250(1), 253-9
CODEN: BIJOAK; ISSN: 0306-3275
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Described is the isolation from **protein** digests, by reversed-phase **HPLC**, of **18O**-labeled and unlabeled **peptides** and their direct anal. by fast-atom-bombardment **mass spectrometry**. Under the conditions used, the **18O** label is retained throughout the **sepn.** and anal., thus permitting assignments of C-terminal **peptides** to be made. Enzyme-catalyzed exchange of label into the terminal carboxy group occurred in some cases without hydrolysis of a **peptide** bond. This effect, which may be exploited to prep. labeled **peptides**, does not prevent application of the method (2 **sep.** digests must then be used). The method was used for the anal. of enzymic partial hydrolyzates of glucagon, insulin, and of several **proteins** produced by expression of recombinant DNA.

IT **9002-07-7, Trypsin**
RL: ANST (Analytical study)
(in C-terminal **peptide** identification by fast-atom-bombardment mass spectroscopy)

IT **14797-71-8, Oxygen-18**, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)

(labeling by, of **proteins**, C-terminal **peptide** identification in relation to)

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 09:08:38 ON 08 AUG 2003)
L8 73 S L7
L9 52 DUP REM L8 (21 DUPLICATES REMOVED)

L9 ANSWER 1 OF 52 MEDLINE on STN
ACCESSION NUMBER: 2003143633 IN-PROCESS
DOCUMENT NUMBER: 22545533 PubMed ID: 12659189
TITLE: Fast-response proteomics by accelerated in-gel digestion of **proteins**.
AUTHOR: Havlis Jan; Thomas Henrik; Sebela Marek; Shevchenko Andrej
CORPORATE SOURCE: Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, D-01307 Dresden, Germany.
SOURCE: ANALYTICAL CHEMISTRY, (2003 Mar 15) 75 (6) 1300-6. Journal code: 0370536. ISSN: 0003-2700.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20030328
Last Updated on STN: 20030328
AB Kinetics of in-gel digestion of **proteins** by modified and native **trypsins** was studied by MALDI TOF **mass spectrometry** using **18O**-labeled **peptides** as internal standards. The effect of the temperature, enzyme concentration, digestion time, and surface area of gel pieces on the yield of digestion products was characterized. Based on the kinetic data, we developed a protocol that enabled the identification of gel-**separated proteins** with 30-min digestion time without compromising the **peptide** yield and the sensitivity compared to conventional protocols that typically rely upon overnight enzymatic cleavage. The accelerated digestion protocol was tested in identification of more than 120 **proteins** from budding and fission yeasts at the subpicomole level.

L9 ANSWER 2 OF 52 MEDLINE on STN
ACCESSION NUMBER: 2003059107 MEDLINE
DOCUMENT NUMBER: 22457203 PubMed ID: 12540831
TITLE: Quantitation of changes in **protein** phosphorylation: a simple method based on stable isotope labeling and **mass spectrometry**.
AUTHOR: Bonenfant Debora; Schmelzle Tobias; Jacinto Estela; Crespo Jose L; Mini Thierry; Hall Michael N; Jenoe Paul
CORPORATE SOURCE: Department of Biochemistry, Biozentrum of the University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2003 Feb 4) 100 (3) 880-5. Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200303
ENTRY DATE: Entered STN: 20030206
Last Updated on STN: 20030319
Entered Medline: 20030318

AB Reversible **protein** phosphorylation plays an important role in many cellular processes. However, a simple and reliable method to measure changes in the extent of phosphorylation is lacking. Here, we present a method to quantitate the changes in phosphorylation occurring in a **protein** in response to a stimulus. The method consists of three steps: (i) enzymatic digestion in H(2)16O or isotopically enriched H(2)**18O** to label individual pools of differentially phosphorylated **proteins**; (ii) affinity selection of phosphopeptides from the combined digests by immobilized metal-affinity chromatography; and (iii) dephosphorylation with alkaline phosphatase to allow for quantitation of changes of phosphorylation by matrix-assisted laser desorption ionization time-of-flight **mass spectrometry**. We applied this strategy to the analysis of the yeast nitrogen permease reactivator **protein** kinase involved in the target of rapamycin signaling pathway. Alteration in the extent of phosphorylation at Ser-353 and Ser-357 could be easily assessed and quantitated both in wild-type yeast cells treated with rapamycin and in cells lacking the SIT4 phosphatase responsible for dephosphorylating nitrogen permease reactivator **protein**. The method described here is simple and allows quantitation of relative changes in the level of phosphorylation in signaling **proteins**, thus yielding information critical for understanding the regulation of complex **protein** phosphorylation cascades.

L9 ANSWER 3 OF 52 MEDLINE on STN
ACCESSION NUMBER: 2003309652 IN-PROCESS
DOCUMENT NUMBER: 22721797 PubMed ID: 12837592
TITLE: **Trypsin** catalyzed (16)O-to-(18)O exchange for comparative proteomics: tandem **mass spectrometry** comparison using MALDI-TOF, ESI-QTOF, and ESI-ion trap **mass spectrometers**.
AUTHOR: Heller Manfred; Mattou Hassan; Menzel Christoph; Yao Xudong
CORPORATE SOURCE: GeneProt Inc., Meyrin, Switzerland.
SOURCE: JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, (2003 Jul) 14 (7) 704-18.
Journal code: 9010412. ISSN: 1044-0305.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20030703
Last Updated on STN: 20030703

AB Quantitative or comparative proteome analysis was initially performed with 2-dimensional gel **electrophoresis** with the inherent disadvantages of being biased towards certain **proteins** and being labor intensive. Alternative

mass spectrometry-based approaches in conjunction with gel-free **protein/peptide separation** have been developed in recent years using various stable isotope labeling techniques. Common to all these techniques is the incorporation, biosynthetically or chemically, of a labeling moiety having either a natural isotope distribution of hydrogen, carbon, oxygen, or nitrogen (light form) or being enriched with heavy isotopes like **deuterium**, (13)C, (18)O, or (15)N, respectively. By mixing equal amounts of a control sample possessing for instance the light form of the label with a heavy-labeled case sample, differentially labeled **peptides** are detected by **mass spectrometric** methods and their intensities serve as a means for direct relative **protein** quantification. While each of the different labeling methods has its advantages and disadvantages, the endoprotease (16)O-to-(18)O catalyzed oxygen exchange at the C-terminal carboxylic acid is extremely promising because of the specificity assured by the enzymatic reaction and the labeling of essentially every protease-derived **peptide**. We show here that this methodology is applicable to complex biological samples such as a subfraction of human plasma. Furthermore, despite the relatively small mass difference of 4 Da between the two labeled forms, corresponding to the exchange of two oxygen atoms by two (18)O isotopes, it is possible to quantify differentially labeled **proteins** on an ion trap **mass spectrometer** with a mass resolution of about 2000 in automated data dependent LC-**MS/MS** acquisition mode. Post column sample deposition on a MALDI target parallel to on-line ESI-MS/MS enables the analysis of the same compounds by means of ESI- and MALDI-MS/MS. This has the potential to increase the confidence in the quantification results as well as to increase the sequence coverage of potentially interesting **proteins** by complementary **peptide** ionization techniques. Additionally the paired y-ion signals in tandem mass spectra of (16)O/(18)O-labeled **peptide** pairs provide a means to confirm automatic **protein** identification results or even to assist de novo sequencing of yet unknown **proteins**.

L9 ANSWER 4 OF 52 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2003068394 EMBASE
TITLE: Stop And Go Extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics.
AUTHOR: Rappsilber J.; Ishihama Y.; Mann M.
CORPORATE SOURCE: M. Mann, Ctr. of Experimental Bioinformatics, Department of Biochemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark. mann@bmb.sdu.dk
SOURCE: Analytical Chemistry, (1 Feb 2003) 75/3 (663-670). Refs: 27
ISSN: 0003-2700 CODEN: ANCHAM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Proteomics is critically dependent on optimal sample preparation.

Particularly, the interface between **protein** digestion and **mass spectrometric** analysis has a large influence on the overall quality and sensitivity of the analysis. We here describe a novel procedure in which a very small disk of beads embedded in a Teflon meshwork is placed as a microcolumn into pipet tips. Termed Stage, for STop And Go Extraction, the procedure has been implemented with commercially available material (C18 Empore Disks (3M, Minneapolis, MN)) as frit and **separation** material. The disk is introduced in a simple and fast process yielding a convenient and completely reliable procedure for the production of self-packed microcolumns in pipet tips. It is held in place free of obstacles solely by the narrowing tip, ensuring optimized loading and elution of analytes. Five disks are conveniently placed in 1 min, adding <0.1 cent in material costs to the price of each tip. The system allows fast loading with low backpressure (> 300 .mu.L/min for the packed column using manual force) while eliminating the possibility of blocking. The loading capacity of C(18)-StageTips (column bed: 0.4 mm diameter, 0.5 mm length) is 2-4 .mu.g of **protein** digest, which can be increased by using larger diameter or stacked disks. Five femtomole of tryptic BSA digest could be recovered quantitatively. We have found that the Stage system is well-suited as a universal sample preparation system for proteomics.

L9 ANSWER 5 OF 52 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003205433 MEDLINE
DOCUMENT NUMBER: 22611070 PubMed ID: 12724941
TITLE: Use of proteomics methodology to evaluate inflammatory **protein** expression in tendinitis.
AUTHOR: Harris Ryan D; Nindl Gabi; Balcavage Walter X; Weiner William; Johnson Mary T
CORPORATE SOURCE: Rose-Hulman Institute of Technology, Terre Haute, IN 47803, USA.
SOURCE: BIOMEDICAL SCIENCES INSTRUMENTATION, (2003) 39 493-9. Journal code: 0140524. ISSN: 0067-8856.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20030503
Last Updated on STN: 20030618
Entered Medline: 20030617

AB In previous studies we established a rat model of acute tendinitis including functional and mechanical measures of healing. Achilles' tendinitis was induced by injection of collagenase, an enzyme that produces localized fiber digestion and edema formation. As quantitative measures of tissue inflammation, hypercellularity and edema were evaluated in injured tendons in comparison with controls. Using the rat tendinitis model, we have applied isotope-coded affinity tag analysis (ICAT) methodology to indicate localized tendon healing by quantitating **protein** expression. This novel proteomics method allows detection of subtle differences in **protein** levels that provide a detailed picture of tendinitis healing. The method involves a new class of chemical linkers used to differentially label cysteine residues from similar **peptides** in control and treated **protein** samples

with heavy (**deuterium** off of backbone) and light (hydrogen off of backbone) ICAT reagents that are otherwise chemically identical. **Proteins** were extracted under liquid nitrogen from control untreated or injured Achilles' tendons 72 hours after collagenase-injection. These **proteins** were digested with endoproteinase Glu-C and **trypsin** and the resulting **peptide** mixtures were evaluated using reverse-phase C18 **HPLC** and Tristricine SDS-polyacrylamide gel **electrophoresis**. The two ICAT-modified **peptide** populations were mixed, affinity-purified and analyzed using microcapillary **liquid chromatography** and electrospray ionization tandem mass-spectroscopy. The process resulted in relative abundance and charge-to-mass ratio data used in conjunction with database searching to identify **proteins** expressed differentially in the two treatment groups. By analyzing different time periods in the healing process, an accurate model of the healing rat tendon can be made.

L9 ANSWER 6 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:253192 BIOSIS
DOCUMENT NUMBER: PREV200300253192
TITLE: Dissection of proteolytic **18O** labeling: Endoprotease-catalyzed 16O-to-**18O** exchange of truncated **peptide** substrates.
AUTHOR(S): Yao, Xudong (1); Afonso, Carlos; Fenselau, Catherine
CORPORATE SOURCE: (1) Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20742, USA: xudong@wam.umd.edu USA
SOURCE: Journal of Proteome Research, (March April 2003) Vol. 2, No. 2, pp. 147-152. print.
ISSN: 1535-3893.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Proteolytic labeling in H2180 has been recently revived as a versatile method for proteomics research. To understand the molecular basis of the labeling process, we have dissected the process into two **separate** events: cleavage of the **peptide** amide bonds and exchange of the terminal carboxyl oxygens. It was demonstrated that both carboxyl oxygens can be catalytically labeled, independent of the cleavage step. Reaction kinetics of the tryptic 16O-to-**18O** exchange of YGGFMR, YGGFMK, and the tryptic digest of apomyoglobin were studied by matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance **mass spectrometry**. A larger KM for the Lys-**peptide** (4400 +- 700 muM), when compared to that of the Arg-**peptide** (KM 1300 +- 300 muM), was mainly responsible for the slower reaction with YGGFMK (kcat/KM 0.64 +- 0.14 muM-1min-1) compared to YGGFMR (kcat/KM 2.6 +- 0.9 muM-1min-1). Multiplexed kinetic studies showed that endoprotease-catalyzed oxygen exchange is a general phenomenon, allowing homogeneous 18O2-coding of a variety of **peptides**. It was demonstrated for the first time that chymotrypsin 18O2-codes **peptides** during proteolysis. On the basis of the analyses reported here, we propose that proteolytic **18O** labeling can be advantageously decoupled from **protein** digestion, and endoproteases can be used in a **separate** step to 18O2-code **peptides** for comparative studies after proteolysis has taken

place.

L9 ANSWER 7 OF 52 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2003:538524 SCISEARCH
THE GENUINE ARTICLE: 690AT
TITLE: **Protein**-folding kinetics and mechanisms studied by pulse-labeling and **mass spectrometry**
AUTHOR: Konermann L (Reprint); Simmons D A
CORPORATE SOURCE: Univ Western Ontario, Dept Chem, London, ON N6A 5B7, Canada (Reprint)
COUNTRY OF AUTHOR: Canada
SOURCE: MASS SPECTROMETRY REVIEWS, (JAN-FEB 2003) Vol. 22, No. 1, pp. 1-26.
Publisher: JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA.
ISSN: 0277-7037.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 217

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The "**protein**-folding problem" refers to the question of how and why a denatured **polypeptide** chain can spontaneously fold into a compact and highly ordered conformation. The classical description of this process in terms of reaction pathways has been complemented by models that describe folding as a biased conformational diffusion on a multidimensional energy landscape. The identification and characterization of short-lived intermediates provide important insights into the mechanism of folding. Pulsed hydrogen/**deuterium** exchange (HDX) methods are among the most powerful tools for studying the properties of kinetic intermediates. Analysis of pulse-labeled **proteins** by **mass spectrometry** (**MS**) provides information that is complementary to that obtained in nuclear magnetic resonance (NMR) studies; NMR data represent an average of entire **protein** ensembles, whereas **MS** can detect co-existing **protein** species. **MS**-based pulse-labeling experiments can distinguish between folding scenarios that involve parallel pathways, and those where folding is channeled through obligatory intermediates. The proteolytic digestion/**MS** technique provides spatially **resolved** information on the HDX pattern of folding intermediates. This method is especially important for **proteins** that are too large to be studied by NMR. Although traditional pulsed HDX protocols are based on quench-flow techniques, it is also possible to use electrospray (ESI) **MS** to analyze the reaction mixture on-line and "quasi-instantaneously" after labeling. This approach allows short-lived **protein** conformations to be studied by their HDX level, their ESI charge-state distribution, and their ligand-binding state. Covalent labeling of free cysteinyl residues provides an alternative approach to pulsed HDX experiments. Another promising development is the use of synchrotron X-rays to induce oxidation at specific sites within a **protein** for studying their solvent accessibility during folding.

L9 ANSWER 8 OF 52 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2003-278215 [27] WPIDS
CROSS REFERENCE: 2003-210067 [20]; 2003-278216 [27]

DOC. NO. NON-CPI: N2003-221053
DOC. NO. CPI: C2003-072581
TITLE: Characterizing **polypeptide** or population of **polypeptides** comprises reacting with lysine reactive agent, optionally amine reactive agent, digesting stage, deactivating reagent stage, removal and recovery stages.
DERWENT CLASS: B04 B05 D16 J04 K08 S03
INVENTOR(S): HAMON, C; JOHNSTONE, R; JOUBERT, R; KUHN, K; NEUMANN, T; SCHMIDT, G; THOMPSON, A
PATENT ASSIGNEE(S): (XZIL-N) XZILLION GMBH & CO KG
COUNTRY COUNT: 100
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002099124	A2	20021212	(200327)*	EN	107

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002099124	A2	WO 2002-GB2601	20020607

PRIORITY APPLN. INFO: EP 2001-304975 20010607
AN 2003-278215 [27] WPIDS
CR 2003-210067 [20]; 2003-278216 [27]
AB WO 200299124 A UPAB: 20030429
NOVELTY - Characterizing **polypeptide** or population of **polypeptides** comprises:
(a) contacting sample comprising one or more **polypeptides** with lysine reactive agent;
(b) optionally reacting the sample with amine reactive reagent;
(c) digesting the sample of **polypeptides** with cleavage reagent;
(d) optionally deactivating cleavage reagent;
(e) removing **peptide** fragments having uncapped or unblocked amino groups; and
(f) recovering the N-terminal **peptide** fragment or fragments.
DETAILED DESCRIPTION - Method (M1) for characterizing a **polypeptide** or a population of **polypeptides** comprises:
(a) contacting a sample comprising one or more **polypeptides** with lysine reactive agent to cap epsilon -amino groups;
(b) optionally reacting the sample with amine reactive reagent to block alpha -amino groups;
(c) digesting the sample of **polypeptides** with a cleavage reagent to produce **peptide** fragments;

(d) optionally deactivating the cleavage reagent;
(e) removing those **peptide** fragments having uncapped or unblocked amino groups; and
(f) recovering the N-terminal **peptide** fragment or fragments.
INDEPENDENT CLAIMS are also included for:
(1) method (M2) for characterizing a **polypeptide** or a population of **polypeptides** comprising:
(a) contacting a sample comprising one or more **polypeptides** with lysine reactive agent to cap epsilon -amino groups;
(b) contacting the resultant capped **polypeptides** with an amine reactive agent which reacts with the unblocked alpha -amino groups at the N-termini of the **polypeptides**;
(c) digesting the sample with a cleavage agent to produce **peptide** fragments;
(d) optionally deactivating the cleavage reagent; and
(e) recovering N-terminal **peptide** that have reacted with the amine reactive agent;
(2) assaying for one or more specific **polypeptides** in a test sample, comprising performing either (M1) or (M2), where the sequence of the **polypeptide** is determined by assaying the resulting N-termini for a predetermined N-terminal sequence of amino acid residues;
(3) characterizing (M3) one or more mixtures of **polypeptides** comprising performing (M1) or (M2) and detecting the **peptides** by **mass spectrometry**;
(4) determining the expression profile of a sample, which comprises characterizing one or more mixtures of **polypeptides** by methods (M1) or (M2) where the sample of step (a) comprises a sub-cellular fraction;
(5) characterizing a **polypeptide** or a population of **polypeptides**, comprising contacting a sample comprising one or more **polypeptides** with a lysine reactive agent comprising a hindered Michael reagent, to attach the agent to epsilon -amino groups;
(6) compound of formula (I);
(7) kit for characterizing a **polypeptide** or population of **polypeptides** comprising:
(i) a lysine reactive agent for capping epsilon -amino groups;
(ii) means for recovering or isolating N-terminal **peptides**;
(iii) optionally an amine reactive reagent for blocking alpha -amino groups; and
(iv) optionally a cleavage reagent for producing peptide fragments; and
(8) use of compound of formula (II) for protecting epsilon -amino groups in peptides and polypeptides.
R1 = pyridyl, quinolyl, pyrazine, pyrimidine or triazine ring structure;
R = H, halo, alkyl or aryl;
Sub = H, halo, hydrocarbon group or an electron withdrawing group;
R1a = alkyl or aryl (including aromatic, cyclic, fused cyclic or heterocyclic groups)
provided that at least one of the R groups comprises a sterically hindering group.

USE - For characterizing polypeptide or a population of polypeptides; For assaying one or more specific polypeptides in a test sample; For determining the expression profile of a sample; (II) is for protecting epsilon -amino groups in peptides and polypeptides against further reaction of the epsilon -amino groups with Edman agents (such as isothiocyanate or isocyanate), capture agents (such as N-hydroxysuccinimidyl biotin) and agents which are capable of reacting with alpha -amino groups (such as acetic acid N-hydroxysuccinimide ester), (all claimed). (I) is a more preferred compound of (II).
Dwg.0/26

L9 ANSWER 9 OF 52 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-590681 [63] WPIDS
DOC. NO. NON-CPI: N2002-468707
DOC. NO. CPI: C2002-167135
TITLE: Identifying a **protein**, by incorporating the selected amino acids into the **proteins** in a sequence-specific manner to enable **protein** identification from the patterns in the mass spectra of proteolytic **peptides**.
DERWENT CLASS: B04 D16 J04 K08 S03
INVENTOR(S): CHEN, X
PATENT ASSIGNEE(S): (CHEN-I) CHEN X; (REGC) UNIV CALIFORNIA
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002055989	A2	20020718	(200263)*	EN	38

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

US 2002146743 A1 20021010 (200269)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002055989	A2	WO 2002-US538	20020111
US 2002146743	A1 Provisional	US 2001-261716P	20010112
		US 2002-43965	20020111

PRIORITY APPLN. INFO: US 2001-261716P 20010112; US 2002-43965 20020111
AN 2002-590681 [63] WPIDS
AB WO 200255989 A UPAB: 20021001
NOVELTY - Identifying (M) a **protein** (I),comprising **separating** (I) from other **proteins**, digesting (I) to form first proteolytic **peptides** (P1), incorporating at least one amino acid 100 % labeled with at least one stable isotope into (I) in a sequence-specific manner, **separating** (I), digesting (I) to form second proteolytic **peptides** (P2) and

comparing the monoisotopic distribution of P2 with P1.

DETAILED DESCRIPTION - Identifying (M) a **protein**, comprising:

(a) **separating** (I) from other **proteins**;

(b) digesting (I), thus forming P1;

(c) acquiring the monoisotopic mass distribution spectrum of the first proteolytic **peptides** and acquiring the m/z values for it, incorporating at least one amino acid 100 % labeled with at least one stable isotope into (I) in a sequence-specific manner;

(d) **separating** (I) bearing at least one labeled amino acid from other **proteins**;

(e) digesting (I) bearing at least one labeled amino acid, thus forming P2, acquiring the monoisotopic mass distribution spectrum of P2 and acquiring the m/z values of it; and

(f) comparing the monoisotopic mass distribution spectrum of P2 with the monoisotopic mass distribution spectrum of P1 to determine the amino acid composition of P1 and P2, where (I) is identified from the m/z values of P1 and P2 and the amino acid composition of P1 and P2.

USE - (M) is useful for identifying a (I) (claimed).

Dwg.0/5

L9 ANSWER 10 OF 52 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN

ACCESSION NUMBER: 2002-627262 [67] WPIDS

DOC. NO. NON-CPI: N2002-496092

DOC. NO. CPI: C2002-176834

TITLE: Analyzing mixtures containing **proteins**, by digesting **proteins** to provide **peptides**, reacting cysteine-containing **proteins** with a reagent, and subjecting the eluted **peptides** to quantitative **mass spectrometry** analysis.

DERWENT CLASS: A96 B04 D16 J04 K08 S03

INVENTOR(S): HEWICK, R M; QIU, Y; WANG, J H

PATENT ASSIGNEE(S): (GEMY) GENETICS INST INC; (GEMY) GENETICS INST LLC

COUNTRY COUNT: 98

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002048717	A2	20020620	(200267)*	EN	44
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2002043385	A	20020624	(200267)		
US 2002164809	A1	20021107	(200275)		
EP 1330654	A2	20030730	(200350)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 2002048717 A2		WO 2001-US50745	20011022
AU 2002043385 A		AU 2002-43385	20011022
US 2002164809 A1	Provisional	US 2000-242643P	20001023
		US 2001-45170	20011022
EP 1330654	A2	EP 2001-989278	20011022
		WO 2001-US50745	20011022

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2002043385	A	Based on	WO 200248717
EP 1330654	A2	Based on	WO 200248717

PRIORITY APPLN. INFO: US 2000-242643P 20001023; US 2001-45170 20011022

AN 2002-627262 [67] WPIDS

AB WO 200248717 A UPAB: 20021018

NOVELTY - Analyzing (M) mixtures containing **proteins** (I), comprising reducing the disulfide bonds in (I) of a sample, thus providing thiol groups in cysteine-containing **proteins** (CP), blocking free thiols with blocking reagent, digesting (I) to provide **peptides**, reacting CP with a reagent, eluting CP and subjecting the eluted **peptides** to quantitative **mass spectrometry** analysis, is new.

DETAILED DESCRIPTION - Analysis (M) of mixtures containing (I), comprising:

(a) reducing the disulfide bonds in the (I) of a sample, thus providing thiol groups in CP;

(b) blocking free thiols with a blocking reagent in the sample;

(c) digesting (I) in the sample to provide **peptides**;

(d) reducing the disulfide bonds in the digested **peptides**, thus providing thiol groups in CP for reaction;

(e) reacting CP in the sample with a reagent, where the reagent comprises a thiol-specific reactive group which is attached to a polymer tag by a linker, where the linker can be differentially labeled with stable isotopes and where the polymer tag forms a covalent bond with CP;

(f) washing the polymer-bound **peptides** to remove non-covalently bound species;

(g) eluting the CP; and

(h) subjecting the eluted **peptides** to quantitative **mass spectrometry** (**MS**) analysis.

INDEPENDENT CLAIMS are also included for the following:

(1) a compound (I) useful for capturing CP, is thiol-specific reactive group attached to a non-biological polymer by a linker; and

(2) a reagent kit (II) for the analysis of (I) by mass spectral analysis that comprises (I).

USE - (M) is useful for analyzing mixtures containing the **proteins**. (I) is useful for capturing CP (claimed). (I) is also useful in a **mass spectrometric** methods for quantitation and identification of one or more **proteins** in a mixture.

Dwg.0/1

L9 ANSWER 11 OF 52 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN

ACCESSION NUMBER: 2002-281071 [32] WPIDS

CROSS REFERENCE: 2002-454319 [48]
DOC. NO. CPI: C2002-082747
TITLE: Determining the absolute quantity of a target **biopolymer**, e.g., a **protein**, comprises determining the ratio of the selected **biopolymer** to its corresponding analog by **mass spectrometric** analysis, and calculating **biopolymer** quantity.
DERWENT CLASS: B04 D16
INVENTOR(S): ESTELL, D A; GANSHAW, G C; PAECH, C; PAECH, S
PATENT ASSIGNEE(S): (GEMV) GENENCOR INT INC; (ESTE-I) ESTELL D A; (GANS-I) GANSHAW G C; (PAEC-I) PAECH C; (PAEC-I) PAECH S
COUNTRY COUNT: 98
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002018644	A2	20020307	(200232)*	EN	41
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2001085063	A	20020313	(200249)		
US 2002123055	A1	20020905	(200260)		
EP 1311707	A2	20030521	(200334)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2002018644	A2		WO 2001-US25884	20010817
AU 2001085063	A		AU 2001-85063	20010817
US 2002123055	A1	Provisional	US 2000-228198P	20000825
			US 2001-932369	20010817
EP 1311707	A2		EP 2001-964178	20010817
			WO 2001-US25884	20010817

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001085063	A	Based on	WO 200218644
EP 1311707	A2	Based on	WO 200218644

PRIORITY APPLN. INFO: US 2000-228198P 20000825; US 2001-932369 20010817
AN 2002-281071 [32] WPIDS
CR 2002-454319 [48]
AB WO 200218644 A UPAB: 20030529
NOVELTY - Determining (M1) the absolute quantity of a **biopolymer** (BP) e.g., a **protein** in a crude solution (S) by:

(a) adding a known quantity of an analog (AG) of BP to (S);
(b) treating BP and AG with fragmenting activity;
(c) **resolving peptide** content;
(d) determining by **mass spectrometric** analysis, the ratio of selected BP to corresponding AG; and
(e) calculating the quantity of BP from the ratio and known quantity of AG.

DETAILED DESCRIPTION - Determining (M1) the absolute quantity of a target **biopolymer** (BP) e.g., a selected **protein** in crude solution (S) comprises:
(a) adding a known quantity of an analog of the target **biopolymer** to the solution;
(b) treating the target **biopolymer** and analog with a fragmenting activity to generate a number of corresponding **biopolymer**-fragment pairs;
(c) **resolving** the **biopolymer**-fragment content of the mixture;
(d) determining by **mass spectrometric** analysis the ratio of a selected target **biopolymer** to its corresponding analog; and
(e) calculating, from the ratio and the known quantity of the analog, the quantity of the target **biopolymer** in the mixture.

INDEPENDENT CLAIMS are also included for the following:
(1) verifying (M2) the presence and, optionally, determining the absolute quantity of a selected putative **biopolymer** in a mixture containing a number of isotope-labeled cellular **biopolymer** from a selected cell type, comprising:
(a) selecting a putative **biopolymer** potentially present in the mixture;
(b) generating a theoretical fragmentation of the putative **biopolymer**;
(c) selecting a theoretical fragment from the theoretical fragmentation;
(d) producing a **biopolymer**-fragment corresponding to the theoretical fragment;
(e) adding a known amount of the produced **biopolymer** -fragment as an internal standard to the mixture;
(f) treating the mixture with a fragmenting activity;
(g) **resolving** the cellular **biopolymer** -fragments along with the internal standard and analyzing the same by **mass spectrometry** to provide a mass spectrograph;
(h) locating a peak pair from the mass spectrograph comprised of a peak representing the internal standard and a peak representing a cellular **biopolymer**-fragment corresponding to the internal standard, thus verifying the presence of the putative **biopolymer**;
(i) optionally, upon verifying the presence of the putative **biopolymer**, determining the ratio of internal standard to its corresponding cellular **biopolymer**-fragment; and
(j) calculating, from the ratio and the known quantity of the internal standard, the absolute quantity of the putative **biopolymer** in the mixture; and
(2) a cell-culture extract (I), derived from a selected microorganism grown on media enriched in a specific isotope, where the extract contains a known amount of a metabolically labeled **biopolymer** determined by a **biopolymer**-

separation technique in combination with mass spectroscopy.

USE - M1 is useful for determining the absolute quantity of a **biopolymer** such as selected **proteins**, **polypeptides** (such as enzymes, **antibodies**, receptors, hormones, growth factors, **antigens**, and ligands) or polynucleotides such as oligonucleotides in a crude solution. (M1) is also useful for determining the identity of a target **biopolymer** fragment in a solution. The method involves:

(a) adding an analog of the target **biopolymer** and the target **biopolymer** to the solution in a selected analog:target ratio;

(b) treating the target **biopolymer** and analog with a fragmenting activity to generate a number of corresponding **biopolymer**-fragment pairs;

(c) **resolving** the **biopolymer**-fragment content of the solution;

(d) identifying by mass spectrometric analysis those biopolymer-fragment pairs that exhibit the selected ratio; and

(e) optionally, determining the biopolymer sequence of the biopolymer-fragment pairs identified.

The solution contains a number of different biopolymers (claimed).

ADVANTAGE - M1 is a straightforward and rapid technique for determining the specific concentration of one or more biopolymers in a mixture.

Dwg.0/7

L9 ANSWER 12 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:72593 BIOSIS
DOCUMENT NUMBER: PREV200300072593
TITLE: Insight into the factors influencing the backbone dynamics of three homologous **proteins**, dendrotoxins I and K, and BPTI: FTIR and time-**resolved** fluorescence investigations.
AUTHOR(S): Hollecker, Michelle (1); Vincent, Michel; Gallay, Jacques; Ruysschaert, Jean-Marie; Goormaghtigh, Erik
CORPORATE SOURCE: (1) Centre de Biophysique Moleculaire, UPR 4301 CNRS, Affiliee Universite Orleans et INSERM, Rue Charles-Sadron, 45071, Orleans Cedex 2, France: holleck@cnrs-orleans.fr France
SOURCE: Biochemistry, (December 24 2002) Vol. 41, No. 51, pp. 15267-15276. print.
ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, combined with hydrogen/**deuterium** exchange technique and time-**resolved** fluorescence spectroscopy, has been used to investigate the changes in structure and dynamics that underlie the thermodynamic stability differences observed for three closely homologous **proteins**: dendrotoxins I and K, and bovine pancreatic **trypsin** inhibitor (BPTI). The experiments were performed on **proteins** under their native state and a modified form, obtained by selective reduction of a disulfide bond at the surface of the molecule, increasing slightly the backbone flexibility without changing the average structure. The

data confirmed the high local as well as global rigidity of BPTI. In **protein** K, the exchange process was slow during the first 2 h of exchange, presumably reflecting a compact three-dimensional conformation, and then increased rapidly, the internal amide protons of the beta-strands exchanging 10-fold faster than in BPTI or **protein** I. The most probable destabilizing element was identified as Pro32, in the core of the beta-sheet. **Protein** I was found to present a 10% more expanded volume than **protein** K or BPTI, and there is a possible correlation between the resulting increased flexibility of the molecule and the lower thermodynamic stability observed for this **protein**. Interestingly, the interior amide protons of the beta-sheet structure were found to be as protected against exchange in **protein** I as in BPTI, suggesting that, although globally more flexible than that of Toxin K or BPTI, the structure of Toxin I could be locally quite rigid. The structural factors suspected to be responsible for the differences in internal flexibility of the two toxins could play a significant role in determining their functional properties.

L9 ANSWER 13 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2002:477502 BIOSIS
DOCUMENT NUMBER: PREV200200477502
TITLE: Correlation of binding-loop internal dynamics with stability and function in potato I inhibitor family: Relative contributions of Arg50 and Arg52 in Cucurbita maxima **trypsin** inhibitor-V as studied by site-directed mutagenesis and NMR spectroscopy.
AUTHOR(S): Cai, Mengli; Gong, Yu-Xi; Wen, Lisa; Krishnamoorthi, Ramaswamy (1)
CORPORATE SOURCE: (1) Department of Biochemistry, Kansas State University, Manhattan, KS, 66506: krish@ksu.edu USA
SOURCE: Biochemistry, (July 30, 2002) Vol. 41, No. 30, pp. 9572-9579. http://pubs.acs.org/journals/bichaw/. print.
ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The side chains of Arg50 and Arg52 at positions P6' and P8', respectively, anchor the binding loop to the **protein** scaffold by means of hydrogen bonds in Cucurbita maxima **trypsin** inhibitor-V (CMTI-V), a potato I family member. Here, we have investigated the relative contributions of Arg50 and Arg52 to the binding-loop flexibility and stability by determining changes in structure, dynamics, and proteolytic stability as a consequence of individually mutating them into an alanine. We have compared chemical shift assignments of main-chain hydrogens and nitrogens, and 1H-1H interresidue nuclear Overhauser effects (NOEs) for the two mutants with those of the wild-type **protein**. We have also measured NMR longitudinal and transverse relaxation rates and **15N**-1H NOE enhancements for all backbone and side-chain NH groups and calculated the model-free parameters for R50A-rCMTI-V and R52A-rCMTI-V. The three-dimensional structures and backbone dynamics of the **protein** scaffold region remain very similar for both mutants, relative to the wild-type **protein**. The flexibility of the binding loop is increased in

both R50A- and R52A-rCMTI-V. In R52A-rCMTI-V, the mean generalized order parameter (<S2>) of the P6-P1 residues of the binding loop (39-44) decreases to 0.68 +- 0.02 from 0.76 +- 0.04 observed for the wild-type **protein**. However, in R50A-rCMTI-V, the flexibility of the whole binding loop increases, especially that of the P1'-P3' residues (45-47), whose <S2> value drops dramatically to 0.35 +- 0.03 from 0.68 +- 0.03 determined for rCMTI-V. More strikingly, S2 values of side-chain NepsilonHs reveal that, in the R50A mutant, removal of the R50 hydrogen bond results in the loss of the R52 hydrogen bond too, whereas in R52A, the R50 hydrogen bond remains unaffected. Kinetic data on **trypsin**-catalyzed hydrolysis of the reactive-site **peptide** bond (P1-P1') suggest that the activation free energy barrier of the reaction at 25 degreeC is reduced by 2.1 kcal/mol for R50A-rCMTI-V and by 1.5 kcal/mol for R52A-rCMTI-V, relative to rCMTI-V. Collectively, the results suggest that although both the P6' and P8' anchors are required for optimal inhibitor function and stability in the potato I family, the former is essential for the existence of the latter and has greater influence on the binding-loop structure, dynamics, and stability.

L9 ANSWER 14 OF 52 MEDLINE on STN
ACCESSION NUMBER: 2002292954 MEDLINE
DOCUMENT NUMBER: 22029211 PubMed ID: 12033290
TITLE: Cis-trans signatures of proline-containing tryptic **peptides** in the gas phase.
AUTHOR: Counterman Anne E; Clemmer David E
CORPORATE SOURCE: Department of Chemistry, Indiana University, Bloomington 47405, USA.
CONTRACT NUMBER: 1R01GM59145 (NIGMS)
SOURCE: ANALYTICAL CHEMISTRY, (2002 May 1) 74 (9) 1946-51. Journal code: 0370536. ISSN: 0003-2700.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200304
ENTRY DATE: Entered STN: 20020530
Last Updated on STN: 20030406
Entered Medline: 20030404

AB High-resolution ion mobility/time-of-flight techniques were used to measure collision cross sections for 968 tryptic digest **peptide** ions obtained from digestion of common **proteins**. Here, we report a mobility signature that aids in identifying proline-containing **peptides** containing 4-10 residues. Of 129 **peptides** (< or = 10 residues in length) in the database that contain proline residues, 57% show multiple **resolved** features in the ion mobility distribution for at least one of the [M + H]+ or [M + **2H**]2+ ions. These multiple features are attributed to different conformations that arise from populations of cis and trans forms of proline. The number of **resolved** peaks in the ion mobility distribution appears to be correlated with the **peptide** ion charge state and the number of proline residues in the **peptide**.

L9 ANSWER 15 OF 52 MEDLINE on STN
ACCESSION NUMBER: 2002367208 MEDLINE
DOCUMENT NUMBER: 22107440 PubMed ID: 12112619

TITLE: Inverse **15N**-metabolic labeling/**mass spectrometry** for comparative proteomics and rapid identification of **protein** markers/targets.

AUTHOR: Wang Y Karen; Ma Zhixiang; Quinn Douglas F; Fu Emil W
CORPORATE SOURCE: Central Technologies, Discovery Research, Novartis Pharmaceuticals Corporation, 556 Morris Avenue, Summit, NJ 07901, USA.. karen.wang@pharma.novartis.com

SOURCE: RAPID COMMUNICATIONS IN MASS SPECTROMETRY, (2002) 16 (14) 1389-97.
Journal code: 8802365. ISSN: 0951-4198.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20020712
Last Updated on STN: 20020824
Entered Medline: 20020823

AB The inverse labeling/**mass spectrometry** strategy has been applied to **protein** metabolic (15)N labeling for gel-free proteomics to achieve the rapid identification of **protein** markers/targets. Inverse labeling involves culturing both the perturbed (by disease or by a drug treatment) and control samples each in two **separate** pools of normal and (15)N-enriched culture media such that four pools are produced as opposed to two in a conventional labeling approach. The inverse labeling is then achieved by combining the normal (14)N-control with the (15)N-perturbed sample, and the (15)N-control with the (14)N-perturbed sample. Both mixtures are then proteolyzed and analyzed by **mass spectrometry** (coupled with on-line or off-line **separation**). Inverse labeling overcomes difficulties associated with **protein** metabolic labeling with regard to isotopic peak correlation and data interpretation in the single-experiment approach (due to the non-predictable/variable mass difference). When two data sets from inverse labeling are compared, **proteins** of differential expression are readily recognized by a characteristic inverse labeling pattern or apparent qualitative mass shifts between the two inverse labeling analyses. MS/MS fragmentation data provide further confirmation and are subsequently used to search **protein** databases for **protein** identification. The methodology has been applied successfully to two model systems in this study. Utilizing the inverse labeling strategy, one can use any **mass spectrometer** of standard unit resolution, and acquire only the minimum, essential data to achieve the rapid and unambiguous identification of differentially expressed **protein** markers/targets. The strategy permits quick focus on the signals of differentially expressed **proteins**. It eliminates the detection ambiguities caused by the dynamic range of detection. Finally, inverse labeling enables the detection of covalent changes of **proteins** responding to a perturbation that one might fail to distinguish with a conventional labeling experiment.

L9 ANSWER 16 OF 52 MEDLINE on STN

ACCESSION NUMBER: 2002458507 MEDLINE
DOCUMENT NUMBER: 22204892 PubMed ID: 12216736
TITLE: Increased proteome coverage for quantitative **peptide** abundance measurements based upon high performance **separations** and DREAMS FTICR **mass spectrometry**.
AUTHOR: Pasa-Tolic Ljiljana; Harkewicz Richard; Anderson Gordon A; Tolic Nikola; Shen Yufeng; Zhao Rui; Thrall Brian; Masselon Christophe; Smith Richard D
CORPORATE SOURCE: Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99352, USA.
CONTRACT NUMBER: CA86340 (NCI)
SOURCE: JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, (2002 Aug) 13 (8) 954-63. Journal code: 9010412. ISSN: 1044-0305.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020910
Last Updated on STN: 20020926
Entered Medline: 20020925

AB A primary challenge in proteome measurements is to be able to detect, identify, and quantify the extremely complex mixtures of **proteins**. The relative abundances of interest span at least six orders of magnitude for mammalian proteomes, and this constitutes an intractable challenge for high throughput proteome studies. We have recently described a new approach, Dynamic Range Enhancement Applied to **Mass Spectrometry** (DREAMS), which is based upon the selective ejection of the most abundant species to expand the dynamic range of Fourier transform ion cyclotron resonanace (FTICR) measurements. The basis of our approach is on-the-fly data-dependent selective ejection of highly abundant species, followed by prolonged accumulation of remaining low-abundance species in a quadrupole external to the FTICR ion trap. Here we report the initial implementation of this approach with high efficiency capillary reverse phase LC **separations** and high magnetic field electrospray ionization FTICR **mass spectrometry** for obtaining enhanced coverage in quantitative measurements for mammalian proteomes. We describe the analysis of a sample derived from a tryptic digest of **proteins** from mouse B16 cells cultured in both natural isotopic abundance and **15N**-labeled media. The FTICR **mass spectrometric** analysis allows the assignment of **peptide** pairs (corresponding to the two distinctive versions of each **peptide**), and thus provides the basis for quantiative measurements when one of the two proteomes in the mixture is perturbed or altered in some fashion. We show that implementation of the DREAMS approach allows assignment of approximately 80% more **peptide** pairs, thus providing quantitative information for approximately 18,000 **peptide** pairs in a single analysis.

L9 ANSWER 17 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:60276 BIOSIS

DOCUMENT NUMBER: PREV200300060276
TITLE: Use of a lectin affinity selector in the search for unusual glycosylation in proteomics.
AUTHOR(S): Xiong, Li (1); Regnier, Fred E.
CORPORATE SOURCE: (1) Department of Chemistry, Purdue University, 1393 Brown Building, West Lafayette, IN, 47907, USA: slentz@purdue.edu USA
SOURCE: Journal of Chromatography B, (25 December 2002) Vol. 782, No. 1-2, pp. 405-418. print.
ISSN: 1387-2273.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The purpose of the work described in this paper was to develop a new approach to the identification of glycoprotein with particular types of glycosylation. The paper demonstrates N-glycosylation sites in a glycoproteins can be identified by (1) proteolysis with **trypsin**, (2) lectin affinity selection, (3) enzymatic deglycosylation with **peptide**-N-glycosidase F (PNGase F) in buffer containing 95% H218O, which generates deglycosylated **peptide** pairs **separated** by 2 or 4 amu, (4) reversed-phase **separation** of the **peptide** mixture and MALDI mass analysis, (5) MS-MS sequencing of the ion pairs, and (6) identification of the parent **protein** through a database search. This process has been tested on the selection of glycopeptides from lactoferrin and mammaglobin, and the identification of the ion pairs of fetuin glycopeptides. Glycosylation sites were identified through PNGase hydrolysis in H218O. During the process of hydrolyzing the conjugate, Asn is converted to an aspartate residue with the incorporation of **18O**. However, PNGase F was observed to incorporate two **18O** into the beta-carboxyl groups of the Asp residue. This suggests that the hydrolysis is at least partially reversible.

L9 ANSWER 18 OF 52 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002199113 MEDLINE
DOCUMENT NUMBER: 21929361 PubMed ID: 11931651
TITLE: Molecular dissection of membrane-transport **proteins: mass spectrometry** and sequence determination of the galactose-H+ symport **protein**, GalP, of Escherichia coli and quantitative assay of the incorporation of [ring-2-**13C**]histidine and (15)NH(3).
AUTHOR: Venter Henrietta; Ashcroft Alison E; Keen Jeffrey N; Henderson Peter J F; Herbert Richard B
CORPORATE SOURCE: Astbury Centre for Structural Molecular Biology, School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.
SOURCE: BIOCHEMICAL JOURNAL, (2002 Apr 15) 363 (Pt 2) 243-52. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020405
Last Updated on STN: 20020517
Entered Medline: 20020516
AB The molecular mass of the galactose-H(+) symport **protein**

GalP, as its histidine-tagged derivative GalP(His)(6), has been determined by electrospray MS (ESI-MS) with an error of <0.02%. One methionine residue, predicted to be present from the DNA sequence, was deduced to be absent. This is a significant advance on the estimation of the molecular masses of membrane-transport **proteins** by SDS/PAGE, where there is a consistent under-estimation of the true molecular mass due to anomalous **electrophoretic** migration. Addition of a size-exclusion chromatography step after Ni(2+)-nitrilotriacetate affinity purification was essential to obtain GalP(His)(6) suitable for ESI-MS. Controlled **trypsin**, **trypsin** +chymotrypsin and CNBr digestion of the **protein** yielded **peptide** fragments suitable for ESI-MS and tandem MS analysis, and accurate mass determination of the derived fragments resulted in identification of 82% of the GalP(His)(6) **protein**. Tandem MS analysis of selected **peptides** then afforded 49% of the actual amino acid sequence of the **protein**; the absence of the N-terminal methionine was confirmed. Matrix-assisted laser-desorption ionization MS allowed identification of one **peptide** that was not detected by ESI-MS. All the **protein/peptide** mass and sequence determinations were in accord with the predictions of amino acid sequence deduced from the DNA sequence of the galP gene. [ring-2-(13)C]Histidine was incorporated into GalP(His)(6) in vivo, and ESI-MS analysis enabled the measurement of a high (80%) and specific incorporation of label into the histidine residues in the **protein**. MS could also be used to confirm the labelling of the **protein** by (15)NH(3) (93% enrichment) and [(19)F]tryptophan (83% enrichment). Such MS measurements will serve in the future analysis of the structures of membrane-transport **proteins** by NMR, and of their topology by indirect techniques.

L9 ANSWER 19 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2002:239786 BIOSIS
DOCUMENT NUMBER: PREV200200239786
TITLE: Proteolytic **18O** labeling for comparative proteomics: Evaluation of endoprotease Glu-C as the catalytic agent.
AUTHOR(S): Reynolds, Kristy J.; Yao, Xudong; Fenselau, Catherine (1)
CORPORATE SOURCE: (1) Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20742: fenselau@wam.umd.edu USA
SOURCE: Journal of Proteome Research, (January February, 2002) Vol. 1, No. 1, pp. 27-33. http://pubs.acs.org/JPR. print. ISSN: 1535-3893.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Recently, proteolytic **18O** labeling has been demonstrated as a promising strategy for comparative proteomic studies (Yao, X.; Freas, A.; Ramirez, J.; Demirev, P. A.; Fenselau, C. Anal. Chem. 2001, 73, 2836-42). In this approach, **protein** mixtures are digested in parallel in H216O and H218O and the ratios of isotopically distinct **peptide** products are measured by **mass spectrometry**. In the initial report from this

laboratory, **trypsin** was shown to catalyze incorporation of two **180** atoms into the carboxyl terminus of each new **peptide** formed by cleavage of the adenovirus proteome. In the present study, a second enzyme, endoprotease Glu-C, is evaluated as an agent for cleavage and labeling. Proteolytic **180** labeling by Glu-C is shown to occur readily with phosphorylated and glycosylated **proteins** and with cysteine-alkylated and disulfide-linked **proteins**. A sequential double-labeling strategy is used to characterize N-linked glycopeptides. Labeled and unlabeled **peptide** pairs are found to coelute chromatographically, and measurements of isotope ratios by nanospray and capillary LC-**MS** are found to be accurate and precise.

L9 ANSWER 20 OF 52 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-216735 [27] WPIDS
DOC. NO. NON-CPI: N2002-166189
DOC. NO. CPI: C2002-066155
TITLE: Encoding (labeling) several **polypeptide** samples for analysis by **mass spectrometry** by cleaving amide backbone of the **polypeptides** in each sample and mass modifying the carboxy terminus of the fragments obtained.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): FIGEYS, D; MANN, M; STEWART, I I; FIGEYS, J M D
PATENT ASSIGNEE(S): (MDSP-N) MDS PROTEOMICS INC; (FIGE-I) FIGEYS D; (MANN-I) MANN M; (STEW-I) STEWART I I
COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001094935	A2	20011213	(200227)*	EN	67
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2001070941	A	20011217	(200229)		
US 2002076817	A1	20020620	(200244)		
EP 1290450	A2	20030312	(200320)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR					

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2001094935	A2		WO 2001-IB1328	20010608
AU 2001070941	A		AU 2001-70941	20010608
US 2002076817	A1	Provisional	US 2000-210496P	20000609
		Provisional	US 2001-293664P	20010525
			US 2001-878750	20010611
EP 1290450	A2		EP 2001-949829	20010608
			WO 2001-IB1328	20010608

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2001070941	A	Based on	WO 200194935
EP 1290450	A2	Based on	WO 200194935

PRIORITY APPLN. INFO: US 2001-293664P 20010525; US 2000-210496P 20000609; US 2001-878750 20010611

AN 2002-216735 [27] WPIDS

AB WO 200194935 A UPAB: 20020429

NOVELTY - Encoding (labeling) several **polypeptide** samples for analysis by **mass spectrometry** by cleaving amide backbone of the **polypeptides** in each sample and mass modifying the carboxy terminus of the fragments obtained

DETAILED DESCRIPTION - Encoding (labeling) (M1) several **polypeptide** samples for analysis by **mass spectrometry** comprises:

(a) cleaving the amide backbone of **polypeptides** of each individual sample to form sub-populations of fragments (I) having carboxy-terminal residues (CTR);

(b) mass-modifying the CTRs of (I) with one of at least two groups of different molecular weight (MWt) to produce several discrete populations of mass-modified (I) which differ in MWt by the addition of the group, where the groups differ in MWt due to inclusion of isotypes of differing MWt, where for each sample, the mass-modification produces several various discrete populations labeled, in a predetermined ratio of at least two groups, where the ratio of each sample is different from the next amongst **polypeptide** samples.

INDEPENDENT CLAIMS are also included for the following:

(1) producing (M2) a **peptide** sample pool (PSP) for analysis by **mass spectrometry** comprising forming a **peptide** digest (PDI) by hydrolyzing a **peptide** sample in the presence of a water containing a volumetric ratio of two members of an isotope, forming a **peptide** digest (PD2) by repeating the above method, where the volumetric ratios of PD1 and PD2 are different from each other; and pooling PD1 and PD2 to form a **peptide** sample;

(2) PSP produced by M2, which is adapted to reveal the **protein** source of each **peptide** in the pool when the pool is analyzed by **mass spectrometry**, comprising PD1 and PD2; and

(3) a software program for high throughput automated analysis of **mass spectrometry** data of **peptide** sample comprises identifying desired **peptides** in a sample with high probability based on their mass data, generating a theoretical natural isotope abundance distribution based on the identification of the **peptides**, subtracting the relative isotopic contribution by each of the labeled states and comparing them in a relative sense to generate the 160/180 of desired ratio.

USE - M1 is useful for quantitating the abundance of a given **polypeptide** present in a sample using **mass spectrometry**. The method comprises carrying out M1, where the mass-modified portions obtained or combined to form a **peptide** sample and subjected to analysis by **mass spectrometry** to mass spectra comprising at least one signal doublet for each fragment where the signal doublet comprises a first

signal and a second signal that shifted a known amount of units from the first signal. The method further comprises determining a signal ratio for at least a fragment pair by relating the difference in signal intensity or area between the first and second signal, where the abundance of the given **polypeptide** is determined from the signal ratio and the known amount of the standard sample of the given **polypeptide**, based on the principle that signal intensity is proportional to **peptide** abundance. M2 is useful for producing PSP for analysis by **mass spectrometry**. The method is useful for tracking the source of every desired **polypeptide**, in a PSP comprising generating PSP by M2 and identifying the source of every desired **polypeptide** utilizing the PSP. PSP is useful for identifying the source of **peptide** subjected as a PSP to analyze by **mass spectrometry**. The method comprises obtaining PSP by M2; subjecting the **peptide** sample to analysis by **mass spectrometry** to generate mass spectra comprising at least one signal doublet for each **peptide** in the sample, where the signal doublet comprises a first signal and second signal shifted a known units from the first signal, where the known units is the difference in MWt between the two isotopes; determining a signal ratio for a given **peptide** by relating the difference in signal intensity or area between the first signal and the second signal; correlating the signal ratio for the given **peptide** with the isotope ratio used to form the given **peptide**, thereby identifying the **protein** source of the given **peptide** (all claimed).

ADVANTAGE - **Peptides** are labeled in terms of spreads based on a probability function for a given relative composition of H2180 in a digestion mixture rather than specific ratios. This reduces the absolute capability of 180 labeling for use as an encryption tool for the purpose of running multiple samples in tandem to reduce analysis time.

Dwg.0/11

L9 ANSWER 21 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:503723 BIOSIS
DOCUMENT NUMBER: PREV200100503723
TITLE: Oxidative modification of tryptophan 43 in the heme vicinity of the F43W/H64L myoglobin mutant.
AUTHOR(S): Hara, Isao; Ueno, Takafumi; Ozaki, Shin-ichi; Itoh, Shinobu; Lee, Keonil; Ueyama, Norikazu; Watanabe, Yoshihito (1)
CORPORATE SOURCE: (1) Institute for Molecular Science, Myodaiji, Okazaki, 444-8585: yoshi@ims.ac.jp Japan
SOURCE: Journal of Biological Chemistry, (September 28, 2001) Vol. 276, No. 39, pp. 36067-36070. print. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The F43W/H64L myoglobin mutant was previously constructed to investigate the effects of electron-rich tryptophan residue in the heme vicinity on the catalysis, where we found that Trp-43 in the mutant was oxidatively modified in the reaction with m-chloroperbenzoic acid (mCPBA). To identify the exact structure of the modified tryptophan in this study, the mCPBA-treated F43W/H64L

mutant has been digested stepwise with Lys-C achromobacter and **trypsin** to isolate two oxidation products by preparative fast **protein liquid chromatography**. The close examinations of the 1H NMR spectra of **peptide** fragments reveal that two forms of the modified tryptophan must have 2,6-disubstituted indole substructures. The **13C** NMR analysis suggests that one of the modified tryptophan bears a unique hydroxyl group in stead of the NH2 group at the amino-terminal. The results together with **mass spectrometry** (**MS**)/**MS** analysis (30 Da increase in mass of Trp-43) indicate that oxidation products of Trp-43 are 2,6-dihydro-2,6-dioxoindole and 2,6-dihydro-2-imino-6-oxoindole derivatives. Our findings is the first example of the oxidation of aromatic carbons by the myoglobin mutant system.

L9 ANSWER 22 OF 52 MEDLINE on STN

ACCESSION NUMBER:	2001255528 MEDLINE
DOCUMENT NUMBER:	21253700 PubMed ID: 11354501
TITLE:	Quantitative analysis of bacterial and mammalian proteomes using a combination of cysteine affinity tags and **15N**-metabolic labeling.
COMMENT:	Comment in: Anal Chem. 2001 May 1;73(9):251A
AUTHOR:	Conrads T P; Alving K; Veenstra T D; Belov M E; Anderson G A; Anderson D J; Lipton M S; Pasa-Tolic L; Udseth H R; Chrisler W B; Thrall B D; Smith R D
CORPORATE SOURCE:	Environmental and Molecular Sciences Laboratory and Molecular Biosciences Department, Pacific Northwest National Laboratory, Richland, Washington 99352, USA.
CONTRACT NUMBER:	CA86340 (NCI)
SOURCE:	ANALYTICAL CHEMISTRY, (2001 May 1) 73 (9) 2132-9. Journal code: 0370536. ISSN: 0003-2700.
PUB. COUNTRY:	United States
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	200106
ENTRY DATE:	Entered STN: 20010618 Last Updated on STN: 20010618 Entered Medline: 20010614

AB We describe the combined use of **15N**-metabolic labeling and a cysteine-reactive biotin affinity tag to isolate and quantitate cysteine-containing **polypeptides** (Cys-**polypeptides**) from Deinococcus radiodurans as well as from mouse B16 melanoma cells. D. radiodurans were cultured in both natural isotopic abundance and **15N**-enriched media. Equal numbers of cells from both cultures were combined and the soluble **proteins** extracted. This mixture of isotopically distinct **proteins** was derivatized using a commercially available cysteine-reactive reagent that contains a biotin group. Following **trypsin** digestion, the resulting modified **peptides** were isolated using immobilized avidin. The mixture was analyzed by capillary reversed-phase **liquid chromatography** (LC) online with ion trap **mass spectrometry** (**MS**) as well as Fourier transform ion cyclotron resonance (FTICR) **MS**. The resulting spectra contain numerous pairs of Cyspolypeptides whose mass difference corresponds to the number of nitrogen atoms present in each of the **peptides**. Designation of Cys-**polypeptide** pairs is also facilitated

by the distinctive isotopic distribution of the **15N** -labeled **peptides** versus their 14N-labeled counterparts. Studies with mouse B16 cells maintained in culture allowed the observation of hundreds of isotopically distinct pairs of **peptides** by LC-FTICR analysis. The ratios of the areas of the pairs of isotopically distinct **peptides** showed the expected 1:1 labeling of the 14N and **15N** versions of each **peptide**. An additional benefit from the present strategy is that the **15N**-labeled **peptides** do not display significant isotope-dependent chromatographic shifts from their 14N-labeled counterparts, therefore improving the precision for quantitating **peptide** abundances. The methodology presented offers an alternate, cost-effective strategy for conducting global, quantitative proteomic measurements.

L9 ANSWER 23 OF 52 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001504379 MEDLINE
DOCUMENT NUMBER: 21156205 PubMed ID: 11258770
TITLE: Characterization of differently processed forms of enolase 2 from Saccharomyces cerevisiae by two-dimensional gel **electrophoresis** and **mass spectrometry**.
AUTHOR: Larsen M R; Larsen P M; Fey S J; Roepstorff P
CORPORATE SOURCE: Department of Biochemistry Molecular Biology, University of Southern Denmark, Odense University.
SOURCE: ELECTROPHORESIS, (2001 Feb) 22 (3) 566-75. Journal code: 8204476. ISSN: 0173-0835.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20010917
Last Updated on STN: 20010917
Entered Medline: 20010913

AB Two-dimensional gel **electrophoresis**, bioinformatics, and **mass spectrometry** are key analysis tools in proteome analysis. The further characterization of post-translational modifications in gel-**separated proteins** relies fully on data obtained by **mass spectrometric** analysis. In this study, stress-induced changes in **protein** expression in Saccharomyces serevisiae were investigated. A total of eleven spots on a silver-stained two-dimensional (2-D) gel were identified by matrix-assisted laser desorption/ionization (MALDI) **peptide** mass mapping to represent C and/or N-terminal processed forms of enolase 2. The processing sites were determined by MALDI **peptide** mass mapping using a variety of **proteolytic enzymes**, by optimizing the sample preparation procedure and by specific labeling of all C-termini derived from in-gel digestion using a buffer containing 16O:**18O** (1:1). Out of eleven processed forms of enolase 2, six were fully characterized and the approximate processing sites identified for the remaining five.

L9 ANSWER 24 OF 52 MEDLINE on STN
ACCESSION NUMBER: 2002044951 MEDLINE
DOCUMENT NUMBER: 21628695 PubMed ID: 11755207
TITLE: Gamma371 Thr-->Ile substitution in the fibrinogen

gammaD domain causes hypofibrinogenaemia.
AUTHOR: Brennan S O; Wyatt J M; Fellowes A P; Dlott J S; Triplett D A; George P M
CORPORATE SOURCE: Molecular Pathology Laboratory, Canterbury Health Laboratories, P.O. Box 151, Christchurch, New Zealand.. steve.brennan@chmeds.ac.nz
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (2001 Dec 17) 1550 (2) 183-8.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020124
Last Updated on STN: 20020213
Entered Medline: 20020212

AB Six members of a family with hypofibrinogenaemia had fibrinogen concentrations ranging from 0.5 to 1.1 mg/ml and, after sequencing the entire coding region and the intron exon boundaries of all three fibrinogen genes, a single heterozygous ACT-->ATT mutation was identified in the gamma gene. This novel mutation was not detected in normal family members or unrelated controls. The gamma371 Thr-->Ile substitution occurs at a conserved threonine in the gammaD domain, but molecules containing the new isoleucine were not present in circulating fibrinogen. The evidence for this was that purified gamma chains had a normal mass of 48375 Da compared to a control of 48374 Da, and tryptic **peptide** maps were entirely normal. The mutation predicts a mass increase of 12 Da in **peptide** T-36, but on mass mapping only the normal [M+**2H**] ion was detected, at 948 m/z. There was no new signal at 954 m/z that would indicate expression of variant chains. Also the normal 948 m/z signal was at the same intensity in digests from the proposita and controls. Crystal structures show a hydrogen bond from the threonine hydroxyl to the main chain and this case suggests this bond is critical in maintaining the structure of the gammaD domain.

L9 ANSWER 25 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:481892 BIOSIS
DOCUMENT NUMBER: PREV200100481892
TITLE: Oxidation and macromolecular arylation in quinol-thioether induced nephrotoxicity.
AUTHOR(S): Lau, Serrine S. (1)
CORPORATE SOURCE: (1) Center for Molecular and Cellular Toxicology, Division of Pharmacology/Toxicology, College of Pharmacy, University of Texas Austin, Austin, TX, 78712: slau@mail.utexas.edu USA
SOURCE: Abstracts of Papers American Chemical Society, (2001) Vol. 222, No. 1-2, pp. TOXI 13. print.
Meeting Info.: 222nd National Meeting of the American Chemical Society Chicago, Illinois, USA August 26-30, 2001 American Chemical Society
. ISSN: 0065-7727.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Glutathione conjugates of hydroquinone (HQ-GSH) produce severe renal

proximal tubular necrosis within the S3 segment of the outer stripe of the outer medulla (OSOM) in rats. Immuno-histochemical staining of kidney slices obtained **2h** after HQ-GSH administration, with **antibodies** directed against adducted **proteins**, revealed that arylated **proteins** were localized in cells within the OSOM which undergo cell necrosis at high dose or subsequently give rise to preneoplastic transformation during chronic low dose exposure. To identify quinol-thioether-derived covalent **protein** adducts which may play a role in HQ-GSH mediated nephrotoxicity and nephrocarcinogenicity, rats were administered 2-(GSyl)HQ (200 mmol/kg, iv). Tissues from the OSOM and cortex were excised and fractionated. Renal **proteins** were subject to SDS-PAGE, and immunostaining revealed several HQ-GSH covalently adducted **proteins**. The corresponding SDS gel bands were excised, digested in gel with **trypsin** and identified by LC-MSMS (Finnigan-MAT LCQ-SEQUEST search program). The identified **proteins** included: GSH S-transferase, g-glutamyl cysteine synthetase, aminopeptidase N, ATP synthase a and b chains, heat shock **proteins**, Na/K transporting ATPase, vitamin D-dependent Ca binding **protein**, thio-specific antioxidant **protein**, catalase, elongation factor 1, enolase, ezrin, fructose-bisphosphate aldolase, various dehydrogenases, transketolase, ribosomal **proteins**, actins and albumin. Identification of these adducted **proteins** may provide further insights into the mechanism(s) of quinol-thioether-mediated nephrotoxicity and nephrocarcinogen-icity.

L9 ANSWER 26 OF 52 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-400193 [34] WPIDS
DOC. NO. NON-CPI: N2000-299759
DOC. NO. CPI: C2000-120936
TITLE: Identifying functional sites in **proteins**, useful for detecting epitopes and ligand binding sites, by complexing with tagged binding partner, then laser irradiation to alter the **protein** close to the tag.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): JOCELYN, H N; LEODOVICO, L L; ILAG, L L; NG, J H
PATENT ASSIGNEE(S): (XERI-N) XERION PHARM GMBH; (XERI-N) XERION PHARM AG; (ILAG-I) ILAG L L; (NGJH-I) NG J H
COUNTRY COUNT: 29
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000031544	A1	20000602	(200034)*	GE	40
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU CA CN IL JP KR NO NZ SG US					
DE 19854196	A1	20000824	(200042)		
AU 2000015567	A	20000613	(200043)		
DE 19854196	C2	20010315	(200115)		
EP 1133697	A1	20010919	(200155)	GE	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
EP 1133697	B1	20020807	(200259)	GE	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
DE 59902301	G	20020912	(200264)		
US 2002132266	A1	20020919	(200264)		
JP 2002531810	W	20020924	(200278)		52

ES 2182584 T3 20030301 (200322)
AU 760221 B 20030508 (200337)

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2000031544	A1		WO 1999-EP9052	19991123
DE 19854196	A1		DE 1998-19854196	19981124
AU 2000015567	A		AU 2000-15567	19991123
DE 19854196	C2		DE 1998-19854196	19981124
EP 1133697	A1		EP 1999-958116	19991123
			WO 1999-EP9052	19991123
EP 1133697	B1		EP 1999-958116	19991123
			WO 1999-EP9052	19991123
DE 59902301	G		DE 1999-502301	19991123
			EP 1999-958116	19991123
			WO 1999-EP9052	19991123
US 2002132266	A1	CIP of	WO 1999-EP9052	19991123
		CIP of	US 2001-856285	20010828
			US 2002-142125	20020509
JP 2002531810	W		WO 1999-EP9052	19991123
			JP 2000-584307	19991123
ES 2182584	T3		EP 1999-958116	19991123
AU 760221	B		AU 2000-15567	19991123

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000015567	A	Based on	WO 200031544
EP 1133697	A1	Based on	WO 200031544
EP 1133697	B1	Based on	WO 200031544
DE 59902301	G	Based on	EP 1133697
		Based on	WO 200031544
JP 2002531810	W	Based on	WO 200031544
ES 2182584	T3	Based on	EP 1133697
AU 760221	B	Previous Publ.	AU 200015567
		Based on	WO 200031544

PRIORITY APPLN. INFO: DE 1998-19854196 19981124
AN 2000-400193 [34] WPIDS
AB WO 200031544 A UPAB: 20021105
NOVELTY - Identifying one or more functional sites in a target **protein** (I) comprises:

(i) complexing (I) with a binding partner (A) that carries a laser-activable tag;

(ii) irradiating the complex (II) formed with laser light to generate free radicals that alter bound (I) selectively at the binding sites; and

(iii) identifying the altered region by a combination of **protein** cleavage and **mass spectrometry** (**MS**).

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for an apparatus for the process comprising an automated system of integrated, independent components.

USE - The method is used to identify any type of functional site in any type of **protein** but particularly

ligand-binding sites and epitopes. It can also be used to investigate pathologically altered **proteins** or oncogenic **proteins**, and to inactivate target pathological **proteins**.

ADVANTAGE - The method does not require knowledge of the three-dimensional structure of (I), and is simple, rapid and automatable. It can identify non-linear or discontinuous epitopes, and determines **protein** function without inactivation of (I) (since the tag is bound to (A) not to (I) itself). The method may be used in vitro or in vivo for studying intra- or extra-cellular **proteins**.

Dwg.0/6

L9 ANSWER 27 OF 52 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2001101725 MEDLINE
DOCUMENT NUMBER: 20532383 PubMed ID: 11080046
TITLE: Characterization of benzoquinone-**peptide** adducts by electrospray **mass spectrometry**.
AUTHOR: Mason D E; Liebler D C
CORPORATE SOURCE: Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona 85721-0207, USA.
CONTRACT NUMBER: ES06694 (NIEHS)
ES10056 (NIEHS)
SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (2000 Oct) 13 (10) 976-82.
Journal code: 8807448. ISSN: 0893-228X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010126

AB Benzoquinone adducts were prepared with model **peptides** to identify characteristic features of adduct fragmentation in tandem **mass spectrometry** (**MS**) experiments. Model **peptides** contained cysteine and had a molecular mass of less than 2 kDa to facilitate **peptide** fragmentation in tandem MS analyses. **Peptides** were adducted with an excess of benzoquinone, and the adducts were analyzed by LC/MS. Adducts were identified by addition of 108 Da to the monoisotopic mass of the **peptide**, except in the case of oxytocin, which formed a bis adduct with addition of 216 Da. Tandem MS experiments were performed on the [M + **2H**](2+) ions and/or the [M + H](+) ions. Sequence information obtained from modified **peptides** was comparable to that of their unmodified counterparts. A unique ion pair **separated** by 141 or 142 Da corresponding to beta-elimination of benzoquinol-S or benzoquinol-SH from a b(n) or y(n) series ion indicated attachment at the sulfur of the cysteine residue. An alternate ion pair of 211 Da corresponded to fragmentation at the **peptide** bond on either side of the adducted cysteine. Enzymatic digestion of BSA and a 2560 Da frog **peptide** with **trypsin** yielded tryptic **peptides**, which were treated with benzoquinone. In addition to ion pairs of 142 and 211 Da, singly and doubly charged tryptic

peptide adducts showed a neutral loss of 142 Da from the precursor. Either one or both ion pairs were present in more than half of all the **peptides** that were examined. The neutral loss of 142 Da was present in all singly charged tryptic **peptide** adducts and in 11 out of 14 doubly charged tryptic **peptide** adducts. The data indicate that reliable detection of benzoquinone-cysteinyl **peptide** adducts requires monitoring of multiple spectral characteristics.

L9 ANSWER 28 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:503178 BIOSIS
DOCUMENT NUMBER: PREV200000503178
TITLE: Expression in Escherichia coli, folding in vitro, and characterization of the carbohydrate recognition domain of the natural killer cell receptor NKR-P1A.
AUTHOR(S): Kogelberg, Heide (1); Lawson, Alexander M.; Muskett, Frederick W.; Carruthers, Robert A.; Feizi, Ten
CORPORATE SOURCE: (1) Glycosciences Laboratory, Imperial College School of Medicine, Northwick Park Campus, Harrow, Middlesex UK
SOURCE: Protein Expression and Purification, (October, 2000) Vol. 20, No. 1, pp. 10-20. print.
ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB NKR-P1A is a homodimeric type II transmembrane **protein** of the C-type lectin family found on natural killer (NK) cells and NK-like T cells and is an activator of cytotoxicity. Toward structure determination by NMR, the recombinant carbohydrate-recognition domain (CRD) of NKR-P1A has been expressed in high-yield in Escherichia coli and folded in vitro. The purified **protein** behaves as a monomer in size-exclusion chromatography and is bound by the conformation-sensitive **antibody**, 3.2.3, indicating a folded structure. A **polypeptide** tag at the N-terminus is selectively cleaved from the CRD after limited **trypsin** digestion in further support of a compact folded structure. The disulfide bonds have been identified by **peptide** mapping and electrospray **mass spectrometry**. These are characteristic of a long form CRD. The 1D NMR spectrum of the unlabeled CRD and the 2D HSQC spectrum of the **15N**-labeled CRD are those of a folded **protein**. Chemical shifts of Halpha and NH protons indicate a considerable amount of beta-strand structure. Successful folding in the absence of Ca2+, coupled with the lack of chemical shift changes upon addition of Ca2+, suggests that the NKR-P1A-CRD may not be a Ca2+-binding **protein**.

L9 ANSWER 29 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 5

ACCESSION NUMBER: 1999:323429 BIOSIS
DOCUMENT NUMBER: PREV199900323429
TITLE: Hepatitis A virus capsid **protein** VP1 has a heterogeneous C terminus.
AUTHOR(S): Graff, Judith (1); Richards, Oliver C.; Swiderek, Kristine M.; Davis, Michael T.; Rusnak, Felicia; Harmon, Shirley A.; Jia, Xi-Yu; Summers, Donald F.;

Ehrenfeld, Ellie
CORPORATE SOURCE: (1) Molecular Hepatitis Section, National Institutes of Health, NIAID, LID, 7 Center Dr., Building 7, Room 200, Bethesda, MD, 20892-0740 USA
SOURCE: Journal of Virology, (July, 1999) Vol. 73, No. 7, pp. 6015-6023.
ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Hepatitis A virus (HAV) encodes a single **polyprotein** which is posttranslationally processed into the functional structural and nonstructural **proteins**. Only one protease, viral protease 3C, has been implicated in the nine **protein** scissions. Processing of the capsid **protein** precursor region generates a unique intermediate, PX (VP1-2A), which accumulates in infected cells and is assumed to serve as precursor to VP1 found in virions, although the details of this reaction have not been determined. Coexpression in transfected cells of a variety of P1 precursor **proteins** with viral protease 3C demonstrated efficient production of PX, as well as VP0 and VP3; however, no mature VP1 **protein** was detected. To identify the C-terminal amino acid residue of HAV VP1, we performed **peptide** sequence analysis by protease-catalyzed (**18O**)H2O incorporation followed by **liquid chromatography** ion-trap microspray tandem **mass spectrometry** of HAV VP1 isolated from purified virions. Two different cell culture-adapted isolates of HAV, strains HM175pE and HM175p35, were used for these analyses. VP1 preparations from both virus isolates contained heterogeneous C termini. The predominant C-terminal amino acid in both virus preparations was VP1-Ser274, which is located N terminal to a methionine residue in VP1-2A. In addition, the analysis of HM175pE recovered smaller amounts of amino acids VP1-Glu273 and VP1-Thr272. In the case of HM175p35, which contains valine at amino acid position VP1-273, VP1-Thr272 was found in addition to VP1-Ser274. The data suggest that HAV 3C is not the protease responsible for generation of the VP1 C terminus. We propose the involvement of host cell protease(s) in the production of HAV VP1.

L9 ANSWER 30 OF 52 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 1999351787 EMBASE
TITLE: Biomonitoring of human exposure to methyl bromide by isotope dilution **mass spectrometry** of **peptide** adducts.
AUTHOR: Sannolo N.; Mamone G.; Ferranti P.; Basile A.; Malorni A.
CORPORATE SOURCE: P. Ferranti, Ctr. Internaz. Serv. Spettrom. Massa, CNR, via P. Castellino 111, 80131 Naples, Italy. ferranti@unina.it
SOURCE: Journal of Mass Spectrometry, (1999) 34/10 (1028-1032).
Refs: 20
ISSN: 1076-5174 CODEN: JMSPFJ
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
035 Occupational Health and Industrial Medicine

046 Environmental Health and Pollution Control
052 Toxicology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A procedure for the determination of haemoglobin adducts formed by exposure to methyl bromide was evaluated as a possible method for the measurement of a biological index of exposure to the alkylating agent. The reaction products after in vitro incubation were used to design the chemical syntheses of deuterated **peptides** corresponding to the tryptic **peptides** where the modified residues had been identified. These **peptides** were used as standards for the quantitative evaluation of real samples. The correlation coefficient was r = 0.998 in the range 2.5-20 ppm. The relative standard deviation was about 3%. Blood samples were digested with **trypsin** and the mixture was analysed by **liquid chromatography**/electrospray **mass spectrometry** through selected ion monitoring of the mass signal relative to the modified **peptides**. The analysis of blood samples from workers exposed to methyl bromide demonstrated the usefulness of this **mass spectrometric**-based method for the monitoring of human exposure to the genotoxic alkylating agent via the synthesis of suitable **peptide** standards. This procedure is the first alternative method to the well established monitoring of N-terminal adducts, the latter not being applicable to all alkylating agents.

L9 ANSWER 31 OF 52 JICST-EPlus COPYRIGHT 2003 JST on STN
ACCESSION NUMBER: 1000131693 JICST-EPlus
TITLE: Analysis of Molecular Surface Structure of HIV **Proteins** by **Deuterium** Labeling and **Peptide** Mapping.
AUTHOR: ISHIKAWA KEIICHIRO
CORPORATE SOURCE: National Inst. Materials and Chemical Res.
SOURCE: J Mass Spectrom Soc Jpn, (1999) vol. 47, no. 6, pp. 397-400. Journal Code: G0046A (Fig. 3, Ref. 4)
ISSN: 1340-8097
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Short Communication
LANGUAGE: Japanese
STATUS: New
AB Acetylation of the amino-groups of HIV-p24 **proteins** with normal acetic anhydride and deuterated acetic anhydride followed by the **mass spectrometric peptide** mapping was carried out for the evaluation of relative reactivities of lysine residues at varying locations. The mild acetylation by normal acetic anhydride giving partially acetylated (h-Ac) lysine residues was followed by the complete deuteroacetylation to label the unreacted lysine residues with trideuteroacetic groups (d-Ac). The relative reactivities of lysine residues were evaluated based on the ratios of h-Ac/d-Ac observed by electrospray ionization **mass spectrometry** of unfractionated tryptic digests of acetylated p24. The relative reactivities of four lysine residues (K158>K170.GEQ.K140>K25) showed a correlation with their solvent accessibilities (SA) derived from the tertiary structure of HIV-p24 predicted by computer modeling and X-rar crystallographic analyses. It is shown that the combination of selective chemical modification and **mass spectrometric peptide** mapping of **proteins** is useful to probe their tertiary structures

derived from X-ray crystallography or NMR spectroscopy. (author abst.)

L9 ANSWER 32 OF 52 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2002165309 EMBASE
TITLE: Electrochemical properties of capillary **electrophoresis**-nanoelectrospray **mass spectrometry**.
AUTHOR: Bateman K.P.
CORPORATE SOURCE: Dr. K.P. Bateman, Merck Frosst Canada Inc., 16711 Trans Canada Highway, Kirkland, Que. H9H 3L1, Canada. Kevin_Bateman@merck.com
SOURCE: Journal of the American Society for Mass Spectrometry, (1999) 10/4 (309-317).
Refs: 38
ISSN: 1044-0305 CODEN: JAMSEF
PUBLISHER IDENT.: S 1044-0305(98)00155-X
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Electrochemical properties are inherent to the techniques of **electrophoresis** and electrospray ionization. Interfacing capillary zone **electrophoresis** (CZE) with electrospray **mass spectrometry** (ESMS) can lead to the observation of oxidized species generated as a result of the electrochemical nature of this coupling. Using a nanoelectrospray (nES) interface combined with CZE, controlled chemical oxidation of **peptides** is demonstrated. The electrolysis of water is used to explain the origin of the chemically oxidized species and this is confirmed using experiments with (18)O labeled water. Identification of the oxidized residues was possible using tandem **mass spectrometry** to sequence the modified **peptides**. Methionine was found to be the most readily oxidized residue, followed by aromatic amino acids. Surprisingly, oxidation of aliphatic residues (leucine) was also observed. Addition of a reducing agent to the CZE buffer was found to reduce, but not eliminate, the extent of oxidation. The electrochemical generation of protons at the electrosprayer was used to assist in the analysis of monophosphate nucleotides. Nucleotides were **separated** as anions followed by detection as [M.+ H](+) ions. Copyright .COPYRGT. 1999 American Society for **Mass Spectrometry**.

L9 ANSWER 33 OF 52 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1999085803 MEDLINE
DOCUMENT NUMBER: 99085803 PubMed ID: 9868912
TITLE: Modification of cysteine residues by alkylation. A tool in **peptide** mapping and **protein** identification.
AUTHOR: Sechi S; Chait B T
CORPORATE SOURCE: The Rockefeller University, New York, New York 10021, USA.. SechiS@erols.com
CONTRACT NUMBER: RR00862 (NCRR)
SOURCE: ANALYTICAL CHEMISTRY, (1998 Dec 15) 70 (24) 5150-8.
Journal code: 0370536. ISSN: 0003-2700.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990209
Last Updated on STN: 20000303
Entered Medline: 19990128

AB Although **mass spectrometric peptide** mapping has become an established technique for the rapid identification of **proteins** isolated by polyacrylamide gel **electrophoresis** (PAGE), the results of the identification procedure can sometimes be ambiguous. Such ambiguities become increasingly prevalent for **proteins** isolated as mixtures or when only very small amounts of the **proteins** are isolated. The quality of the identification procedure can be improved by increasing the number of **peptides** that are extracted from the gel. Here we show that cysteine alkylation is required to ensure maximal coverage in matrix-assisted laser desorption/ionization time-of-flight **mass spectrometry** (MALDI-TOF **MS**) **peptide** mapping of **proteins** isolated by PAGE. In the described procedure, alkylation was performed prior to **electrophoresis** to avoid the adventitious formation of acrylamide adducts during **electrophoresis**. In this way, homogeneous alkylation was obtained with three different alkylating reagents (4-vinylpyridine, iodoacetamide, acrylamide). Cysteine alkylation was also used as a tool for the identification of cysteine-containing **peptides** . Using a 1:1 mixture of unlabeled acrylamide and **deuterium** -labeled acrylamide ([2,3,3'-D3]acrylamide), the **proteins** of interest were alkylated prior to **electrophoretic separation**. **Peptide** mixtures produced by **trypsin** digestion of the resulting **protein** bands were analyzed by MALDI-TOF MS, and the cysteine content of the **peptides** was inferred from the isotopic distributions. The cysteine content information was readily obtained and used to improve the **protein** identification process.

L9 ANSWER 34 OF 52 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1998:423923 SCISEARCH
THE GENUINE ARTICLE: ZQ419
TITLE: Local structure and dynamics in **proteins** characterized by hydrogen exchange and **mass spectrometry**
AUTHOR: Smith D L (Reprint)
CORPORATE SOURCE: UNIV NEBRASKA, DEPT CHEM, LINCOLN, NE 68588 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY-MOSCOW, (MAR 1998) Vol. 63, No. 3, pp. 285-293.
Publisher: PLENUM PUBL CORP, CONSULTANTS BUREAU, 233 SPRING ST, NEW YORK, NY 10013.
ISSN: 0006-2979.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 60
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Amide hydrogen exchange rates, determined by NMR spectroscopy, have become an important tool that is often used to investigate structure and dynamics of small **proteins**. Recent developments in **mass spectrometry** and sample handling methods make possible measurement of **deuterium** levels at **peptide** amide linkages in **polypeptides** . The ability to make these measurements has led to development of the **protein** fragmentation/**mass spectrometry** approach for determining amide hydrogen exchange rates in short segments of intact **proteins** following their incubation in D2O. partially deuterated **proteins** are proteolytically fragmented into **peptides** whose molecular weights are determined by on-line **liquid chromatography/mass spectrometry**. **Deuterium** levels, which are determined from the molecular weights of the peptic fragments, can be used to determine amide hydrogen exchange rates. Details of the **protein** fragmentation/**mass spectrometry** approach, along with a brief review of the theory of amide hydrogen exchange, are described. The ability to detect and locate minor structural differences in **proteins** by the **protein** fragmentation/**mass spectrometry** approach is illustrated using oxidized and reduced cytochrome c. These results show that oxidation of iron has little effect on the N- and C-terminal regions, but significantly destabilizes the interior regions of cytochrome c. The ability to detect localized unfolding in large **proteins** is illustrated with aldolase that was equilibrated in acid. Despite the success achieved by NMR spectroscopy for determining amide hydrogen exchange rates, **mass spectrometry** is, advantageous because it permits studies of large **proteins**, requires only picomoles of **protein**, and provides a direct measure of structural heterogeneity.

L9 ANSWER 35 OF 52 MEDLINE on STN
ACCESSION NUMBER: 1998222543 MEDLINE
DOCUMENT NUMBER: 98222543 PubMed ID: 9561756
TITLE: **Liquid chromatographic** -high-resolution **mass spectrometric** and tandem **mass spectrometric** identification of synthetic **peptides** using electrospray ionization.
AUTHOR: D'Agostino P A; Hancock J R; Provost L R; Semchuk P D; Hodges R S
CORPORATE SOURCE: Defence Research Establishment Suffield, Alberta, Canada.
SOURCE: JOURNAL OF CHROMATOGRAPHY. A, (1998 Mar 20) 800 (1) 89-100.
Journal code: 9318488.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 19980520
Entered Medline: 19980514
AB **Liquid chromatography**-high-resolution

electrospray **mass spectrometry** (LC-ESI-**MS**) was investigated for the identification of known and unknown synthetic **peptides** in a research effort designed to evaluate the applicability of this and complementary **MS** techniques for **peptide** characterization and identification. The monoisotopic molecular masses of five related **peptides** with molecular masses between 2000 and 2500 u were acquired with a resolution of 3000 (10% valley). Under narrow and wide mass range magnetic sector scanning conditions monoisotopic molecular mass errors were typically in the 10-20 and 30-40 ppm range, respectively. Tryptic maps were generated for each **peptide** following LC-ESI-MS analysis and collisionally activated dissociation (CAD) in the ESI interface resulted in the production of characteristic product ions that enabled amino acid sequencing of the tryptic fragments. Unknown identification was demonstrated during analysis of an incomplete synthetic **peptide** reaction mixture. The synthesis of an 18 amino acid **peptide**, LTTAVKKVLTTGLPALIS, was not successful. In its place were six unknown **peptides** that were identified on the basis of monoisotopic molecular mass and amino acid sequence data. The monoisotopic molecular masses of these unknowns were determined to within 10-20 ppm with a resolution of 3500 (10% valley). Amino acid sequences for the six **peptides** were generated during ESI-MS-MS analysis. Finally two synthetic **peptides** differing only by the incorporation of a **13C** at leucine were analysed with a resolution of 6000 (10% valley) to confirm that the isotopic distributions were consistent with theoretical expectations.

L9 ANSWER 36 OF 52 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 97:285403 SCISEARCH
THE GENUINE ARTICLE: WR180
TITLE: Hydrogen exchange electrospray ionization **mass spectrometry** studies of substrate and inhibitor binding and conformational changes of Escherichia coli dihydrodipicolinate reductase
AUTHOR: Wang F; Blanchard J S (Reprint); Tang X J
CORPORATE SOURCE: YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT BIOCHEM, 1300 MORRIS PK AVE, BRONX, NY 10461 (Reprint); YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT BIOCHEM, BRONX, NY 10461
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (1 APR 1997) Vol. 36, No. 13, pp. 3755-3759.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 18
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Escherichia coli dihydrodipicolinate reductase is one of seven enzymes in the succinylase pathway of bacterial L-lysine biosynthesis. The binding of NADH, a substrate, and 2,6-pyridinedicarboxylate, an inhibitor, to the recombinant, overexpressed enzyme has been analyzed using hydrogen/

deuterium exchange and electrospray ionization/**mass spectrometry**. NADH binding reduces the extent of **deuterium** exchange, as does the subsequent binding of 2,6-pyridinedicarboxylate. Pepsin digestion of the deuterated enzyme and enzyme-inhibitor complex coupled with **liquid chromatography/mass spectrometry** has allowed the identification of four **peptides** whose **deuterium** exchange slows considerably upon the binding of the substrate or inhibitor. Two of these **peptides** represent regions known or thought to bind NADH and 2,6-pyridinedicarboxylate. Two additional **peptides** are located at the interdomain hinge region and are proposed to be exchangeable in the ''open'', catalytically inactive, conformation but are nonexchangeable in the ''closed'', catalytically active conformation formed after NADH and 2,6-pyridinedicarboxylate binding and domain closure. These studies provide a clear example of a catalytically essential domain movement in this enzyme.

L9 ANSWER 37 OF 52 MEDLINE on STN
ACCESSION NUMBER: 97348592 MEDLINE
DOCUMENT NUMBER: 97348592 PubMed ID: 9204576
TITLE: Rapid 'de novo' **peptide** sequencing by a combination of nanoelectrospray, isotopic labeling and a quadrupole/time-of-flight **mass spectrometer**.
AUTHOR: Shevchenko A; Chernushevich I; Ens W; Standing K G; Thomson B; Wilm M; Mann M
CORPORATE SOURCE: Protein & Peptide Group, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.
SOURCE: RAPID COMMUNICATIONS IN MASS SPECTROMETRY, (1997) 11 (9) 1015-24.
Journal code: 8802365. ISSN: 0951-4198.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970922
Last Updated on STN: 19970922
Entered Medline: 19970909

AB **Protein** microanalysis usually involves the sequencing of gel-**separated proteins** available in very small amounts. While **mass spectrometry** has become the method of choice for identifying **proteins** in databases, in almost all laboratories 'de novo' **protein** sequencing is still performed by Edman degradation. Here we show that a combination of the nanoelectrospray ion source, isotopic end labeling of **peptides** and a quadrupole/ time-of-flight instrument allows facile read-out of the sequences of tryptic **peptides**. Isotopic labeling was performed by enzymatic digestion of **proteins** in 1:1 16O/**18O** water, eliminating the need for **peptide** derivatization. A quadrupole/time-of-flight **mass spectrometer** was constructed from a triple quadrupole and an electrospray time-of-flight instrument. Tandem mass spectra of **peptides** were obtained with better than 50 ppm mass accuracy and resolution routinely in excess of 5000. Unique and error tolerant identification of yeast **proteins** as well as the sequencing

of a novel **protein** illustrate the potential of the approach. The high data quality in tandem mass spectra and the additional information provided by the isotopic end labeling of **peptides** enabled automated interpretation of the spectra via simple software algorithms. The technique demonstrated here removes one of the last obstacles to routine and high throughput **protein** sequencing by **mass spectrometry**.

L9 ANSWER 38 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 7

ACCESSION NUMBER: 1997:125199 BIOSIS
DOCUMENT NUMBER: PREV199799431702
TITLE: Biological fate of sulphur mustard: In vitro alkylation of human haemoglobin by sulphur mustard.
AUTHOR(S): Black, R. M. (1); Harrison, J. M.; Read, R. W.
CORPORATE SOURCE: (1) Chemical Biological Defence Sector, Protection Life Sci. Div., Defence Eval. Res. Agency, Porton Down, Salisbury SP4 0JQ UK
SOURCE: Xenobiotica, (1997) Vol. 27, No. 1, pp. 11-32. ISSN: 0049-8254.
DOCUMENT TYPE: Article
LANGUAGE: English

AB 1. Human blood was incubated in vitro with a 1:1 mixture of (35S,12C-4)- and (**13C**-4)-sulphur mustard. Alkylated globin, containing the 2-hydroxyethylthioethyl (HETE) moiety, was isolated from the blood incubate following lysis of the erythrocytes and acidification with HCl in isopropanol. 2. The alkylated globin was hydrolysed with Pronase E to give a digest containing alkylated amino acids and alkylated dipeptides. A number of these were partially purified by **hplc** and identified by gc-**ms** and lc-**ms**. 3. The alkylated globin was hydrolysed with **trypsin** to give a digest containing alkylated **peptides**. Ten of these were partially purified by **hplc**, tentatively identified by lc-electrospray **mass spectrometry**, and the sequences and sites of alkylation determined using lc-electrospray tandem **mass spectrometry**. 4. (2-Hydroxyethylthioethyl)glutathione was also shown to be present in the pronase and **trypsin** digests of alkylated globin. 5. N-terminal valine, on both the alpha and beta chains, and histidine residues were identified as the key sites of interaction for targeting as biological markers of sulphur mustard poisoning.

L9 ANSWER 39 OF 52 MEDLINE on STN DUPLICATE 8

ACCESSION NUMBER: 1998051076 MEDLINE
DOCUMENT NUMBER: 98051076 PubMed ID: 9390710
TITLE: Quantitative analysis of exogenous **peptides** in plasma using immobilized enzyme cleavage and gas chromatography-**mass spectrometry** with negative ion chemical ionization.
AUTHOR: Marquez C D; Lee M L; Weintraub S T; Smith P C
CORPORATE SOURCE: College of Pharmacy, University of Texas at Austin, 78712, USA.
CONTRACT NUMBER: DA 08088 (NIDA)
GM41828 (NIGMS)
SOURCE: JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL SCIENCES AND APPLICATIONS, (1997 Oct 24) 700 (1-2) 9-21. Journal code: 9714109. ISSN: 1387-2273.

PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980206

AB A method is presented for the analysis of **peptides** in plasma at picomole to femtomole levels. **Peptides** are isolated from plasma by solid-phase extraction, the **peptide** of interest is purified by reversed-phase high-performance **liquid chromatography** (**HPLC**) and selectively digested using immobilized **trypsin** or chymotrypsin to yield specific di- or tripeptides. These di- and tripeptides are esterified using heptafluorobutyric anhydride, alkylated with pentafluorobenzyl bromide, then quantified by gas chromatography-**mass spectrometry** with negative ion chemical ionization. This method has been evaluated for a model synthetic heptapeptide, using a **deuterium** labeled analog as an internal standard. The half-life of the heptapeptide in human plasma was found to be 2 min. Extraction efficiencies of a tritiated **peptide** of similar size to the heptapeptide, [3H]DSLET, from plasma using either C18 or strong cation-exchange columns were 85+/-3 and 70+/-2%, respectively. Quantitation of fragments from the heptapeptide indicated that the analysis was linear from 1-50 ng of the heptapeptide per ml of plasma. This method was subsequently employed for pharmacokinetic studies of the biologically active **peptide** Met-enkephalin-Arg-Gly-Leu, where linearity was obtained from 50 to 1000 ng/ml in rat plasma. This method demonstrated negligible side reaction by-products due to autolysis, and has potential for extensive use given the wide availability of gas chromatography-**mass spectrometry**.

L9 ANSWER 40 OF 52 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 96348786 EMBASE
DOCUMENT NUMBER: 1996348786
TITLE: High-resolution multistage MS, MS2, and MS3 matrix-assisted laser desorption/ionization FT-ICR mass spectra of **peptides** from a single laser shot.
AUTHOR: Solouki T.; Pasa-Tolic L.; Jackson G.S.; Guan S.; Marshall A.G.
CORPORATE SOURCE: Ctr. Interdiscipl. Magn. Resonance, National High Magnetic Field Lab., Florida State University, 1800 East Paul Dirac Drive,Tallahassee, FL 32310, United States
SOURCE: Analytical Chemistry, (1996) 68/21 (3718-3725). ISSN: 0003-2700 CODEN: ANCHAM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB By combined and repeated use of sustained off-resonance irradiation (SORI) for ion dissociation, stored waveform inverse Fourier

transform (SWIFT) waveforms for ion isolation, and ion axialization and remeasurement techniques, we obtain for the first time **MS**, MS2, and MS3 FT-ICR mass spectra from **peptide** ions (enzymatic digest products of horse cytochrome c) produced from a single laser shot. The successive fragmentation of gas-phase ions detected from the same initial batch of ions increases the sensitivity of analysis of trace amounts of biological samples in structural **mass spectrometry**, and fragment identification is facilitated by resolution of **carbon-13** isotopic distributions. The method is illustrated by analyses of subfemtomole amounts of crudely purified samples of tryptic digest solutions of horse cytochrome c and bovine cytochrome c. The high-resolution primary ion mass spectrum, along with the collision-induced dissociation (CID) and **MS**(n) capabilities of FT-ICR, help to determine the primary amino acid sequence of the fragment ions beyond what is obtained from enzymatic digestion alone, without prior chromatographic **separation** and purification.

L9 ANSWER 41 OF 52 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 96:100954 SCISEARCH
THE GENUINE ARTICLE: TR178
TITLE: AMIDE HYDROGEN-EXCHANGE DETERMINED BY **MASS -SPECTROMETRY** - APPLICATION TO RABBIT MUSCLE ALDOLASE
AUTHOR: ZHANG Z Q; POST C B; SMITH D L (Reprint)
CORPORATE SOURCE: UNIV NEBRASKA, DEPT CHEM, LINCOLN, NE, 68588 (Reprint); UNIV NEBRASKA, DEPT CHEM, LINCOLN, NE, 68588; PURDUE UNIV, DEPT MED CHEM, W LAFAYETTE, IN, 47907
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (23 JAN 1996) Vol. 35, No. 3, pp. 779-791.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 68
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **protein** fragmentation/**mass spectrometry** method described by Zhang and Smith [(1993) **Protein** Sci. 2, 522-531] has been extended to measure amide hydrogen exchange rates in rabbit muscle aldolase, a homotetramer with M(r) = 157 000. Following a period of **deuterium** exchange, the partially deuterated **protein** was proteolytically fragmented into **peptides** whose **deuterium** contents were determined by directly coupled **HPLC** fast atom bombardment **mass spectrometry**. Hydrogen exchange rates were determined for amide hydrogens located in short segments derived from 85% of the aldolase backbone. Isotopic exchange rate constants spanning the range from 100 to 0.001 h(-1) were determined for the exchange-in times-used in this study (2.5 min to 44 h). The exchange rates for amide hydrogens located within short segments differed by as much as 10(4), demonstrating that local structural features dramatically affect the isotopic exchange rates in large **proteins**. A high level of correlation between the slowing of hydrogen exchange and intramolecular hydrogen bonding in aldolase was found. An

exception to this correlation occurs at the subunit interface, where the amide hydrogens in one **peptide** segment with few amide hydrogen bonds have slower exchange rates than expected, suggesting that the amide hydrogens in this region are effectively shielded from the deuterated solvent. Isotope patterns observed for most **peptides** were binomial, indicating that hydrogen exchange proceeds through the EX2 mechanism (uncorrelated exchange). However, bimodal isotope patterns were found for **peptides** derived from three short segments of aldolase (including residues 58-64, 279-283, and 326-337), suggesting structural differences in these regions. A high level of correlation was found between crystallographic B-factors and amide hydrogen exchange rates, suggesting an isotopic exchange mechanism involving localized low-amplitude, high-frequency motions that do not require collective motion of many residues. From a methodology viewpoint, these results demonstrate that the combination of **protein** fragmentation with **mass spectrometry** is a useful method for determining the rates at which amide hydrogens located over major portions of large **proteins** undergo isotopic exchange.

L9 ANSWER 42 OF 52 MEDLINE on STN
ACCESSION NUMBER: 97161736 MEDLINE
DOCUMENT NUMBER: 97161736 PubMed ID: 9008840
TITLE: Complete 1H and **13C** resonance assignments of a 21-amino acid glycopeptide prepared from human serum transferrin.
AUTHOR: Lu J; van Halbeek H
CORPORATE SOURCE: Complex Carbohydrate Research Center, University of Georgia, Athens 30602-4712, USA.
CONTRACT NUMBER: P41-RR05351 (NCRR)
SOURCE: CARBOHYDRATE RESEARCH, (1996 Dec 24) 296 1-21. Journal code: 0043535. ISSN: 0008-6215.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970306
Last Updated on STN: 19970306
Entered Medline: 19970224

AB A 21-amino acid glycopeptide (Gp21) was isolated and purified in multi-milligram yields from commercially available human serum transferrin (HSTF) by a combination of tryptic digestion, Con A affinity chromatography, and reverse phase **HPLC**. The **peptide** chain of Gp21 contains a single N-glycosylation site to which a diantennary oligosaccharide is attached. The amino acid sequence and the glycan primary structure of Gp21 have been verified by **peptide** sequencing, electrospray **mass spectrometry**, and one-dimensional 1H NMR spectroscopy. Different glycoforms were found for the glycan of Gp21 derived from two different batches of commercial HSTF. These glycoforms differ from one another in the number of NeuAc residues (ranging from 0 to 2) and/or the number of Gal residues (ranging from 1 to 2). As for the monogalacto species, in the two-dimensional nuclear Overhauser effect (NOE) spectrum of Gp21, interglycosidic NOEs were observed between Man4 in the alpha (1-->3) branch and the terminal GlcNAc beta (1-->2) residue. No interglycosidic NOE was observed between Man4' in the alpha (1-->6) branch and the terminal GlcNAc residue.

These observations indicate that the terminal GlcNAc residue in the minor glycoforms of Gp21 is exclusively located in the alpha (1-->3) branch of the Gp21 glycan. The occurrence of such a carbohydrate structure in HSTF has not been reported before. The 1H and **13C** NMR spectra of Gp21 have been completely assigned by two-dimensional homonuclear and heteronuclear spectroscopy. The close similarity of the 1H and **13C** chemical shift values for the Gp21 glycan with the respective values for the **peptide**-free diantennary oligosaccharide (Wieruszeski et al., Glycoconjugate J., 6 (1989) 183-194) indicates that the 1H and **13C** chemical shifts of the diantennary oligosaccharide are not perturbed by the presence of the Gp21 **peptide** fragment. The complete 1H and **13C** resonance assignments and the full characterization of the primary structure of Gp21 will permit us to study the conformation and dynamics of the N-linked diantennary oligosaccharides while covalently attached to a **polypeptide** fragment.

L9 ANSWER 43 OF 52 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1995:494376 BIOSIS
DOCUMENT NUMBER: PREV199598508676
TITLE: Purification of recombinant human rhinovirus 14 3C protease expressed in Escherichia coli.
AUTHOR(S): Birch, Gary M. (1); Black, Thomas; Malcolm, Sandra K.; Lai, Mei T.; Zimmerman, Ron E.; Jaskunas, S. Richard
CORPORATE SOURCE: (1) Infectious Disease Research, Lilly Research Lab., Eli Lilly and Co., Indianapolis, IN 46285 USA
SOURCE: Protein Expression and Purification, (1995) Vol. 6, No. 5, pp. 609-618.
ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English

AB A gene encoding the human rhinovirus 14 (HRV14) sequence for expression of the viral **polypeptide protein** DELTA-3ABC was inserted into a plasmid driven by the heat-inducible bacteriophage lambda-P-L promoter. The coding sequence was also inserted into a pET vector for expression in the T7 system to produce **13C, 15N**-labeled **protein**. The expressed HRV14 3C protease (3C-pro) autocatalytically cleaved itself from the **polyprotein** DELTA-3ABC, and the mature HRV14 3C-pro partitioned predominantly, in the case of the T7 system, in the insoluble fraction and exclusively, in the case of the P-L system, in the insoluble fraction. The insoluble HRV14 3C-pro was solubilized in urea and purified using anion- and cation-exchange chromatography. The protease was refolded/activated and further purified using a size-exclusion column. HRV14 3C-pro was purified to gt 90% homogeneity as shown by SDS-PAGE and to 95% by **HPLC**. A continuous fluorescence assay was developed which utilized an intramolecularly quenched 9-amino-acid substrate. The substrate anthranilic acid (Anc)-Thr-Leu-Phe-Gln-Gly-Pro-Val-(p-NO-2)-Phe-Lys mimicked the natural 2C/3A cleavage site (Thr-Leu-Phe-Gln-Gly-Pro-Val-Tyr-Phe) using an N-terminal anthranilic acid donor group on one side of the scissile bond (Gln/Gly) and a p-NO-2-Phe acceptor group at the P4 position. Measured by the fluorescence assay, HRV14 3C-pro had a K-m of 300 mu-M for the substrate.

L9 ANSWER 44 OF 52 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 95331462 MEDLINE
DOCUMENT NUMBER: 95331462 PubMed ID: 7607393
TITLE: Cyanopeptolin S, a sulfate-containing depsipeptide from a water bloom of Microcystis sp.
AUTHOR: Jakobi C; Oberer L; Quiquerez C; Konig W A; Weckesser J
CORPORATE SOURCE: Institut fur Biologie II-Mikrobiologie, Albert-Ludwigs-Universitat Freiburg, Germany.
SOURCE: FEMS MICROBIOLOGY LETTERS, (1995 Jun 15) 129 (2-3) 129-33.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950828
Last Updated on STN: 19950828
Entered Medline: 19950816

AB A new sulfated, cyclic depsipeptide, called cyanopeptolin S, from Microcystis sp. was isolated from a water bloom in the Auensee/Leipzig (Germany). The depsipeptide had a relative molecular mass of 925 and contained L-arginine, L-threonine, L-isoleucine, N-methyl-L-phenylalanine, a L-glutamic acid-delta-aldehyde ring system and a sulfated D-configurated glyceric acid as a side chain. The structure was elucidated by means of two-dimensional 1H and **13C** nuclear magnetic resonance spectroscopy, fast atom bombardment mass spectroscopy. Fourier transformed infrared spectroscopy and combined gas-**liquid chromatography/mass spectrometry**. Cyanopeptolin S inhibited **trypsin** with an IC50 < or = 0.2 micrograms ml-1.

L9 ANSWER 45 OF 52 MEDLINE on STN
ACCESSION NUMBER: 95178612 MEDLINE
DOCUMENT NUMBER: 95178612 PubMed ID: 7873661
TITLE: Molecular characterization of surface topology in **protein** tertiary structures by amino-acylation and **mass spectrometric peptide** mapping.
AUTHOR: Glocker M O; Borchers C; Fiedler W; Suckau D; Przybylski M
CORPORATE SOURCE: Fakultat fur Chemie, Universitat Konstanz, Germany.
SOURCE: BIOCONJUGATE CHEMISTRY, (1994 Nov-Dec) 5 (6) 583-90.
Journal code: 9010319. ISSN: 1043-1802.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950419
Last Updated on STN: 19950419
Entered Medline: 19950404

AB Amino-acetylation and -succinylation reactions in combination with **mass spectrometric peptide** mapping of tryptic **peptide** mixtures have been employed for surface

topology-probing of lysine residues in bovine ribonuclease A, lysozyme, and horse heart myoglobin as model **proteins** of different surface structures. Direct molecular weight determinations identifying the precise number of acyl groups in partially modified **proteins** were obtained by electrospray and 252Cf-plasma desorption **mass spectrometry**. Electrospray mass spectra of multiply protonated molecular ions and **deuterium** exchange experiments provided a relative conformational characterization of **protein** derivatives and enabled the direct determinations of intact, partially acylated heme-myoglobin derivatives. Tryptic **peptide** mapping analysis, using plasma desorption and fast atom bombardment **mass spectrometry**, ascertained by **mass spectrometric** characterization of **HPLC-separated** modified **peptides**, yielded the exact identification of acylation sites. Relative reactivities of the amino acylation were derived from the **peptide** mapping data and from quantitative estimations of modified **peptides** upon acetylation/trideuteroacetylation and provided direct correlations with the relative surface accessibilities of lysine-epsilon-amino groups taken from X-ray crystallographic structure data of the **proteins**. The reactive lysine-41 residue in ribonuclease A which is part of the substrate binding site was directly identified from the **mass spectrometric** data. These results indicate tertiary structure-selective acylation combined with **mass spectrometric peptide** mapping as an efficient approach for the molecular characterization of surface topology and reactive fundamental lysine residues in **proteins**.

L9 ANSWER 46 OF 52 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 94:369769 SCISEARCH
THE GENUINE ARTICLE: ND335
TITLE: AMIDE HYDROGEN-EXCHANGE AND **MASS-SPECTROMETRY** - A PROBE OF HIGH-ORDER STRUCTURE IN **PROTEINS**
AUTHOR: SMITH D L (Reprint); ZHANG Z Q; LIU Y Q
CORPORATE SOURCE: PURDUE UNIV, DEPT MED CHEM, W LAFAYETTE, IN, 47907 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: PURE AND APPLIED CHEMISTRY, (JAN 1994) Vol. 66, No. 1, pp. 89-94.
ISSN: 0033-4545.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: ENGLISH
REFERENCE COUNT: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A new analytical method which facilitates **peptide** amide hydrogen as a tool for detecting conformational changes and probing high-order structure in large **proteins** is described. Following a period of **deuterium** exchange-in, the **protein** is placed in slow exchange conditions and fragmented into **peptides** with pepsin. The **peptides** are analyzed by directly-coupled **HPLC** fast atom bombardment **mass spectrometry** to determine their **deuterium** content. Results presented here demonstrate that this method can be used to determine rate constants for

peptide amide hydrogen exchange, and to detect the thermal denaturing of cytochrome c.

L9 ANSWER 47 OF 52 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 94:91011 SCISEARCH
THE GENUINE ARTICLE: MU064
TITLE: PROBING HIGH-ORDER STRUCTURE OF **PROTEINS** BY FAST-ATOM-BOMBARDMENT **MASS-SPECTROMETRY**
AUTHOR: LIU Y Q; SMITH D L (Reprint)
CORPORATE SOURCE: PURDUE UNIV, DEPT MED CHEM, W LAFAYETTE, IN, 47907 (Reprint); PURDUE UNIV, DEPT MED CHEM, W LAFAYETTE, IN, 47907
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, (JAN 1994) Vol. 5, No. 1, pp. 19-28. ISSN: 1044-0305.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: ENGLISH
REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB During the past decade, numerous investigations have demonstrated that the rate at which amide hydrogens located at **peptide** linkages undergo isotopic exchange is a sensitive probe of the high order structure and dynamics of **proteins**. The present investigation demonstrates that microbore high-performance **liquid chromatography** (**HPLC**) continuous-flow fast-atom bombardment **mass spectrometry** (FABMS) can be used to accurately quantify **deuterium** located at **peptide** linkages in short segments of large **proteins**. This result is important because it demonstrates the feasibility of using **mass spectrometry** as a tool for studying the high order structure and dynamics of large **proteins**. Following a period of **deuterium** exchange-in, a **protein** was placed into slow-exchange conditions and fragmented into **peptides** with pepsin. The digest was analyzed by continuous-flow **HPLC** FABMS to determine the molecular weights of the **peptides**, from which the number of deuterons located at the **peptide** linkages could be deduced. The **HPLC** step was used both to fractionate the **peptides** according to their hydrophobicities and to remove through back-exchange all **deuterium** except that located at **peptide** amide linkages. This approach has been applied to alpha-crystallin, a lens **protein** composed of two gene products with monomer molecular weights of 20 kDa and an aggregate molecular weight approaching 1000 kDa. Results from this study show that some of the **peptide** amide hydrogens in alpha A-crystallin exchange very rapidly ($k > 10$ $h(-1)$) while others exchange very slowly ($k < 10(-3)$ $h(-1)$). The ability not only to detect that a conformational change has occurred, but also to identify the specific regions within the **protein** where the change occurred, was demonstrated by measuring changes in the exchange rates within these regions as the **deuterium** exchange-in temperature was increased from 10 to 80 degrees C.

L9 ANSWER 48 OF 52 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

ACCESSION NUMBER: 93:215375 SCISEARCH
THE GENUINE ARTICLE: KV394
TITLE: DETERMINATION OF AMIDE HYDROGEN-EXCHANGE BY **MASS-SPECTROMETRY** - A NEW TOOL FOR **PROTEIN**-STRUCTURE ELUCIDATION
AUTHOR: ZHANG Z Q; SMITH D L (Reprint)
CORPORATE SOURCE: PURDUE UNIV, DEPT MED CHEM & PHARMACOGNOSY, W LAFAYETTE, IN, 47907
COUNTRY OF AUTHOR: USA
SOURCE: PROTEIN SCIENCE, (APR 1993) Vol. 2, No. 4, pp. 522-531.
ISSN: 0961-8368.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A new method based on **protein** fragmentation and directly coupled microbore high-performance **liquid chromatography**-fast atom bombardment **mass spectrometry** (**HPLC**-FABMS) is described for determining the rates at which **peptide** amide hydrogens in **proteins** undergo isotopic exchange. Horse heart cytochrome c was incubated in D2O as a function of time and temperature to effect isotopic exchange, transferred into slow exchange conditions (pH 2-3, 0-degrees-C), and fragmented with pepsin. The number of **peptide** amide deuterons present in the proteolytic **peptides** was deduced from their molecular weights, which were determined following analysis of the digest by **HPLC**-FABMS. The present results demonstrate that the exchange rates of amide hydrogens in cytochrome c range from very rapid ($k > 140$ h-1) to very slow ($k < 0.002$ h-1). The **deuterium** content of specific segments of the **protein** was determined as a function of incubation temperature and used to indicate participation of these segments in conformational changes associated with heating of cytochrome c. For the present **HPLC**-FABMS system, approximately 5 nmol of **protein** were used for each determination. Results of this investigation indicate that the combination of **protein** fragmentation and **HPLC**-FABMS is relatively free of constraints associated with other analytical methods used for this purpose and may be a general method for determining hydrogen exchange rates in specific segments of **proteins**.

L9 ANSWER 49 OF 52 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 93104550 MEDLINE
DOCUMENT NUMBER: 93104550 PubMed ID: 1467549
TITLE: An investigation of fragmentation mechanisms of doubly protonated tryptic **peptides**.
AUTHOR: Tang X J; Boyd R K
CORPORATE SOURCE: Institute for Marine Biosciences, National Research Council, Halifax, Nova Scotia, Canada.
SOURCE: RAPID COMMUNICATIONS IN MASS SPECTROMETRY, (1992 Nov) 6 (11) 651-7.
Journal code: 8802365. ISSN: 0951-4198.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199301
ENTRY DATE: Entered STN: 19930212
Last Updated on STN: 19930212
Entered Medline: 19930122

AB **Peptides** formed as reaction products, of specific hydrolysis of **proteins** by **trypsin**, are characterized by a basic residue (Arg or Lys) at the C-terminus, which facilitates formation of abundant [M + **2H**]2+ ions under electrospray or ionspray conditions. These doubly charged ions readily dissociate upon collisional activation to y" and b fragment ions which are mass complements of one another. The suggestion that these fragments are formed by direct charge-**separation** dissociations must contend with the observation that the y" intensities are generally appreciably larger than those of their b counterparts. However, it is shown that this can be accounted for by a greater susceptibility of the b ions to undergo further dissociation to smaller fragments such as immonium ions. In addition no evidence could be found to support alternative mechanisms, including dissociative electron capture, for which equal intensities of the two fragment ion series are not obligatory. Initial protonation at the N-terminus was shown to be required for formation of these [M + **2H**]2+ ions via its suppression by mono-acetylation at the N-terminus. These findings, and others concerning formation of [y"']2+ fragments, are consistent with extensions of published mechanisms for formation of b and of y" fragments from singly protonated **peptides**, via charge-site-induced cleavages and intramolecular proton transfers between nitrogen atoms, respectively.

L9 ANSWER 50 OF 52 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 93072849 MEDLINE
DOCUMENT NUMBER: 93072849 PubMed ID: 1443554
TITLE: A method for determination of N-glycosylation sites in glycoproteins by collision-induced dissociation analysis in fast atom bombardment **mass spectrometry**: identification of the positions of carbohydrate-linked asparagine in recombinant alpha-amylase by treatment with **peptide** -N-glycosidase F in **18O**-labeled water.
AUTHOR: Gonzalez J; Takao T; Hori H; Besada V; Rodriguez R; Padron G; Shimonishi Y
CORPORATE SOURCE: Institute for Protein Research, Osaka University, Japan.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1992 Aug 15) 205 (1) 151-8. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 20000303
Entered Medline: 19921216

AB Previously, a combined use of fast atom bombardment (FAB) **mass spectrometry** and **peptide** N-glycosidase F, an enzyme that cleaves the beta-aspartylglycosylamine linkage of Asn-linked carbohydrates, was

successfully applied to identification of N-glycosylation sites in a glycoprotein with the known or DNA-derived sequence (S. A. Carr and G. D. Roberts, 1986, Anal. Biochem. 157, 396-406). Here, we extended the method for easier identification of N-glycosylation sites in a glycoprotein even with unknown sequence. The glycoprotein is digested with **peptide**-N-glycosidase F in buffer containing 40 at% H2 **18O**, to yield a deglycosylated **protein** whose carbohydrate-linked Asn residues are converted to Asp partly labeled with **18O** at their beta-carboxyl group during this digestion. The deglycosylated **protein** is further digested with **proteolytic enzymes** in an appropriate buffer prepared with normal water, and then **peptides** are **separated** on a reversed-phase column by **HPLC**. **Peptides** in which carbohydrate-linked Asn has been converted to Asp show a pair of signals ([M + 1]+ and [M + 3]+) in FAB mass spectra due to the partial incorporation of **18O** into the beta-carboxyl groups of Asp residues, while the other **peptides** show normal isotopic ion distributions. Thus, both formally N-glycosylated **peptides** and, using collision-induced dissociation analysis, N-glycosylation sites can be identified. The application of the present method to the determination of N-glycosylation sites in a recombinant glycoprotein, Bacillus licheniformis alpha-amylase, is described.

L9 ANSWER 51 OF 52 MEDLINE on STN DUPLICATE 12

ACCESSION NUMBER: 91023455 MEDLINE
DOCUMENT NUMBER: 91023455 PubMed ID: 2221371
TITLE: Optimization of immobilized enzyme hydrolysis combined with high-performance **liquid chromatography**/thermospray **mass spectrometry** for the determination of neuropeptides.
AUTHOR: Voyksner R D; Chen D C; Swaisgood H E
CORPORATE SOURCE: Analytical and Chemical Sciences, Research Triangle Institute, Research Triangle Park, North Carolina 27709.
CONTRACT NUMBER: 5 R01 DA 04202 (NIDA)
SOURCE: ANALYTICAL BIOCHEMISTRY, (1990 Jul) 188 (1) 72-81. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199011
ENTRY DATE: Entered STN: 19910117
Last Updated on STN: 19910117
Entered Medline: 19901107

AB Peptidases, including chymotrypsin, thermolysin, **trypsin**, V8 protease, and carboxypeptidases A, B, and Y, were immobilized for use in conjunction with **HPLC**/thermospray MS for the analysis of neuropeptides. The optimal operating conditions for each immobilized enzyme bioreactor were determined. Optimal hydrolysis usually occurred at the highest percentage of aqueous solution in the mobile phase at pH 7-8 and 40-50 degrees C. Often post-**HPLC** column addition of aqueous solutions before the bioreactor could improve activity and thermospray sensitivity without changing the **HPLC separation**. Enzymatic hydrolysis requirements were compatible under conditions for

HPLC separation and thermospray MS detection of the selected neuropeptides. Synthetic alpha-, beta-, and gamma-endorphins were the primary neuropeptides used to evaluate on-line immobilized enzyme bioreactor/MS. **HPLC** followed by peptidase hydrolysis produced characteristic hydrolysis products for confirming the **peptides'** identity using thermospray MS detection. Furthermore, the **peptide** formed from enzymatic hydrolysis resulted in a MS ion current 10-40 times higher than that of the [M + **2H**]2+ ion for unhydrolyzed beta-endorphin. The increased sensitivity achieved for detecting the hydrolysis products permits detection and quantitation of synthetic **peptides** down to 800 fmol.

L9 ANSWER 52 OF 52 MEDLINE on STN
ACCESSION NUMBER: 81000304 MEDLINE
DOCUMENT NUMBER: 81000304 PubMed ID: 7407084
TITLE: Changes in pKa values of individual histidine residues of human hemoglobin upon reaction with carbon monoxide.
AUTHOR: Ohe M; Kajita A
SOURCE: BIOCHEMISTRY, (1980 Sep 16) 19 (19) 4443-50. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198011
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19801125

AB We performed the **deuterium**-exchange reaction on human hemoglobin in its carbon monoixy and deoxy forms at various pH values and 36.5 degrees C. **Peptides** containing only one histidine residue were **separated** from tryptic and chymotryptic digests of the deuterated hemoglobin, except for two **peptides** which contained the alpha-87 and alpha-89 and the beta-116 and beta-117 histidine residues, respectively. The pseudo-first-order rate constant for the exchange reaction of each histidin residue was measured by using the **mass spectrometric** method. We obtained the following results. The pKa values for the alpha-20, alpha-89, and beta-146 histidine residues in deoxyhemoglobin decreased significantly, while that for the beta-143 histidine residue increased significantly on ligation. The pseudo-first-order rate constants were virtually zero for the alpha-45, alpha-58, alpha-87, beta-63, and beta-92 histidine residues which are linked with a heme group, and also for the alpha-122 histidine residue which is buried at the alpha 1 beta 1 contact in the hemoglobin molecule. No change was detected in the pKa values on ligation for the other histidine residues in deoxyhemoglobin.

FILE 'HOME' ENTERED AT 09:10:54 ON 08 AUG 2003

(FILE 'MEDLINE' ENTERED AT 09:23:09 ON 08 AUG 2003)

L10	30564	SEA	FILE=MEDLINE	ABB=ON PLU=ON	"SPECTRUM ANALYSIS, MASS"/CT
L11	90013	SEA	FILE=MEDLINE	ABB=ON PLU=ON	"CHROMATOGRAPHY, HIGH PRESSURE LIQUID"/CT
L12	30198	SEA	FILE=MEDLINE	ABB=ON PLU=ON	ELECTROPHORESIS/CT
L13	7039	SEA	FILE=MEDLINE	ABB=ON PLU=ON	L10 AND (L11 OR L12)
L14	31451	SEA	FILE=MEDLINE	ABB=ON PLU=ON	TRYPSIN/CT
L15	259	SEA	FILE=MEDLINE	ABB=ON PLU=ON	L13 AND L14
L16	3546	SEA	FILE=MEDLINE	ABB=ON PLU=ON	BIOPOLYMERS/CT
L17	118761	SEA	FILE=MEDLINE	ABB=ON PLU=ON	PROTEINS/CT
L18	82	SEA	FILE=MEDLINE	ABB=ON PLU=ON	POLYPROTEINS/CT
L19	72092	SEA	FILE=MEDLINE	ABB=ON PLU=ON	PEPTIDES/CT
L20	48553	SEA	FILE=MEDLINE	ABB=ON PLU=ON	ANTIGENS/CT
L21	59976	SEA	FILE=MEDLINE	ABB=ON PLU=ON	ANTIBODIES/CT
L22	65	SEA	FILE=MEDLINE	ABB=ON PLU=ON	L15 AND (L16 OR L17 OR L18 OR L19 OR L20 OR L21)
L23	19	SEA	FILE=MEDLINE	ABB=ON PLU=ON	L22 AND METHODS/CT

L23 ANSWER 1 OF 19 MEDLINE on STN
AN 2002287829 MEDLINE
TI Differential isotopic mass splitting as a mass spectrometric tool for identifying protease substrates.
AU McKeown Stephen C; Heudi Olivier; Kay Corinne; Marshall Peter
SO RAPID COMMUNICATIONS IN MASS SPECTROMETRY, (2002) 16 (11) 1054-8. Journal code: 8802365. ISSN: 0951-4198.
AB A method is described whereby stable isotopic signatures were partially incorporated into both termini of a peptide sequence giving rise to a characteristic cluster of four peaks in the mass spectral analysis. Cleavage of this peptide by a protease between the labeled positions generates two fragments both displaying their own individual signature peaks. The event of protease cleavage of the peptide was monitored by the changes in clusters within the spectrum. We believe that this technique could be used to aid the discovery of new cleavage substrates for proteases. Additionally, the analysis can be automated with dedicated software designed to select and interpret the data since all peaks of interest contain predefined signatures and can be easily distinguished from background noise.

L23 ANSWER 2 OF 19 MEDLINE on STN
AN 2001462707 MEDLINE
TI Selective analysis of phosphopeptides within a protein mixture by chemical modification, reversible biotinylation and mass spectrometry.
AU Adamczyk M; Gebler J C; Wu J
SO RAPID COMMUNICATIONS IN MASS SPECTROMETRY, (2001) 15 (16) 1481-8. Journal code: 8802365. ISSN: 0951-4198.
AB A new method combining chemical modification and affinity purification is described for the characterization of serine and threonine phosphopeptides in proteins. The method is based on the conversion of phosphoserine and phosphothreonine residues to S-(2-mercaptoethyl)cysteinyl or beta-methyl-S-(2-mercaptoethyl)cysteinyl residues by beta-elimination/1,2-ethanedithiol addition, followed by reversible biotinylation of the modified proteins. After trypsin digestion, the biotinylated peptides were affinity-isolated and enriched, and subsequently

subjected to structural characterization by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Database searching allowed for automated identification of modified residues that were originally phosphorylated. The applicability of the method is demonstrated by the identification of all known phosphorylation sites in a mixture of alpha-casein, beta-casein, and ovalbumin. The technique has potential for adaptations to proteome-wide analysis of protein phosphorylation.

L23 ANSWER 3 OF 19 MEDLINE on STN
AN 2000317021 MEDLINE
TI A novel Ser O-glucuronidation in acidic proline-rich proteins identified by tandem mass spectrometry.
AU Jonsson A P; Griffiths W J; Bratt P; Johansson I; Stromberg N; Jornvall H; Bergman T
SO FEBS LETTERS, (2000 Jun 16) 475 (2) 131-4.
Journal code: 0155157. ISSN: 0014-5793.
AB Human acidic proline-rich salivary protein PRP-1 and its C-terminally truncated form PRP-3 were analyzed by electrospray tandem mass spectrometry. Post-translational modifications were detected and characterized. A pyroglutamic acid residue was demonstrated at the N-terminus, Ser-8 and Ser-22 were shown to be phosphorylated and an O-linked glucuronic acid conjugation was identified. The latter modification was located to Ser-17 and found to be present in approximately 40% of the polypeptides.

L23 ANSWER 4 OF 19 MEDLINE on STN
AN 2000191517 MEDLINE
TI Bioconjugation of ribonuclease A: a detailed chromatographic and mass spectrometric analysis of chemical modification by a cross-linking reagent.
AU Mendez T J; Johnson J V; Nichols L S; Lang G H; Eyler J R; Powell D H; Richardson D E
SO BIOCONJUGATE CHEMISTRY, (2000 Mar-Apr) 11 (2) 182-94.
Journal code: 9010319. ISSN: 1043-1802.
AB The modification of ribonuclease A with the heterobifunctional cross-linker, 4-succinimdyloxycarbonyl-methyl-alpha-[2-pyridyldithio]-toluene (SMPT) is described. RNase A has 11 potential sites of modification by the SMPT reagent. Tracking the two-dimensional separation and proteolytic digestion of SMPT-modified RNase A with ESI/FTICR-MS and HPLC/ESI/QIT-MS demonstrates the detailed information about number of SMPT modifications and sites of modification that can be obtained by application of these techniques. Analysis of native and modified RNase A tryptic digests by ESI/FTICR-MS resulted in the identification of the sites of modification. Semiquantitative results of the reactivity of certain lysine residues toward the coupling reagent SMPT are presented. Two sites (lysines 1 and 37) are highly reactive, while three sites (lysines 41, 61, and 104) appear to be unreactive toward SMPT under the conditions used. Experimental results demonstrate that quantitative comparison of relative intensities of peptide sequences of different charge states is not possible. No correlation was found between number of basic residues and sensitivity to detection. Digestion of the modified and unmodified RNase A by subtilisin followed by examination by HPLC/ESI/QIT-MS and MS(n) enabled further investigation of modification on lysines 1 and 7, including modification at the

epsilon- and alpha-amino positions on lysine 1.

L23 ANSWER 5 OF 19 MEDLINE on STN
AN 1998197090 MEDLINE
TI Visualization of proteins by modification of lysines, cysteines, and phosphorylated serines facilitates sample preparation for microsequencing.
AU Hsi K L; O'Neill S A; Dupont D R; Yuan P M
SO ANALYTICAL BIOCHEMISTRY, (1998 Apr 10) 258 (1) 38-47.
Journal code: 0370535. ISSN: 0003-2697.
AB A procedure for visualization and sensitive detection of protein during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent sample preparation for sequence analysis is described. This procedure utilizes either fluorescent or visible tags for certain amino acids in protein molecules, e.g., lysines modified with dansyl/dabsyl chloride and cystines/cysteines or phosphorylated serines modified with iodoacetamidofluorescein (I-15) after proper sample pretreatments. Modifications are performed prior to SDS-PAGE, eliminating the need for fixing, staining, and destaining as required for the conventional procedures. After electrophoresis, the fluorescent or visible bands are excised from the gel, homogenized in microcentrifuge tubes, and soaked in an appropriate buffer to release the separated proteins into solution. Enzymatic digestion can then be carried out in solution for better efficiency of digestion and recovery. The subsequent HPLC mapping and collection of protein digests are performed on PE Applied Biosystems Model 173A MicroBlotter. The separated peptides containing tagged amino acids are visible on the PVDF membrane and can be excised for direct sequence analysis. This approach has been employed for selectively isolating the lysine, cysteine, or phosphorylated serine containing peptides using model proteins. The sequencing results of the peptides generated from premodified proteins demonstrate that this approach facilitates sample preparation for microsequence analysis at low picomole level. Overall recoveries of 20-30% by sequencing initial yields have been achieved using our model proteins.

L23 ANSWER 6 OF 19 MEDLINE on STN
AN 97414229 MEDLINE
TI Combined mass spectrometric methods for the characterization of human hemoglobin variants localized within alpha T9 peptide: identification of Hb Villeurbanne alpha 89 (FG1) His-->Tyr.
AU Deon C; Prome J C; Prome D; Francina A; Groff P; Kalmes G; Galacteros F; Wajcman H
SO JOURNAL OF MASS SPECTROMETRY, (1997 Aug) 32 (8) 880-7.
Journal code: 9504818. ISSN: 1076-5174.
AB Mutation-induced amino acid exchanges occurring on the large T9 peptide of the alpha-chain of human hemoglobin (residues 62-90) are difficult to identify. Despite their high m/z value (around m/z 3000), collision-induced dissociation spectra of liquid secondary ion mass spectrometrically generated protonated alpha T9 peptides were performed successfully. In parallel electrospray mass spectrometry (MS) was used both to measure the molecular mass of the intact proteins and to determine the number of protonatable sites in the alpha T9 peptides. Peptide ladder sequencing using carboxypeptidase digestions and analysis of the truncated peptides by matrix-assisted laser desorption ionization time-of-flight MS

confirmed the interpretation. This set of methods allowed the characterization of three hemoglobin variants, with amino acid exchanges located in the alpha T9 part of the sequence. Two of them, Hb Aztec [alpha 76(EF5) Met-->Thr] and Hb M-Iwate [alpha 87(F8) His-->Tyr] were already known. The third [alpha 89(FG1) His-->Tyr] was novel and named Hb Villeurbanne.

L23 ANSWER 7 OF 19 MEDLINE on STN
AN 96377168 MEDLINE
TI S-pyridylethylation of intact polyacrylamide gels and in situ digestion of electrophoretically separated proteins: a rapid mass spectrometric method for identifying cysteine-containing peptides.
AU Moritz R L; Eddes J S; Reid G E; Simpson R J
SO ELECTROPHORESIS, (1996 May) 17 (5) 907-17.
Journal code: 8204476. ISSN: 0173-0835.
AB In-gel proteolytic digestion of acrylamide-gel separated proteins is a method widely used for generating peptide fragments for the purpose of identifying proteins by Edman degratation, tandem mass spectrometry, and peptide-mass fingerprinting. However, it is well recognised for disulfide-bonded proteins electrophoresed under reducing conditions that if no precautions are taken to minimise disulfide bond formation during protein digestion or peptide isolation, complex peptide maps can result. Here, we describe an improved method for in-gel protein digestion. It consists of first reducing and S-pyridylethylating Coomassie Brilliant Blue R-250-stained proteins immobilised in the whole gel slab with dithiothreitol and 4-vinylpyridine, excising the individual stained and alkylated proteins, and then digesting them in situ in the gel matrix with trypsin or Achromobacter lyticus protease I. Peptide fragments generated in this manner are extracted from the gel piece and purified to homogeneity by a rapid (< or = 12 min) reversed-phase high performance liquid chromatography (HPLC) procedure, based upon conventional silica supports. Recoveries of peptides are increased by S-pyridylethylation of acrylamide-immobilised proteins prior to in-gel digestion. Further, the levels of gel-related contaminants, which otherwise result in suppression of sample signals during electrosprayionisation mass spectrometry, are greatly reduced by the reduction/alkylation step. Additionally, we demonstrate that S-beta-(4-pyridylethyl)-cysteine containing peptides can be readily identified during reversed-phase HPLC by absorbance at 254 nm, and during electrospray ionisation tandem mass spectrometry by the appearance of a characteristic-pyridylethyl fragment ion of 106 Da. The position of cysteine residues in a sequence can be determined as phenylthiohydantoin S-beta-(4-pyridylethyl)-cysteine during Edman degradation, and by tandem mass spectrometry.

L23 ANSWER 8 OF 19 MEDLINE on STN
AN 96377167 MEDLINE
TI Enhanced in situ gel digestion of electrophoretically separated proteins with automated peptide elution onto mini reversed-phase columns.
AU Eckerskorn C; Grimm R
SO ELECTROPHORESIS, (1996 May) 17 (5) 899-906.
Journal code: 8204476. ISSN: 0173-0835.
AB An improved method for the generation and automated isolation of internal peptides by in situ gel digestion of electrophoretically separated proteins is described. To enhance the sensitivity of the

method, and to reduce the amount of sample handling steps, we have automated the extraction procedure of peptides after protein cleavage in a sodium dodecyl sulfate (SDS) gel matrix. The excised protein-containing polyacrylamide bands or spots are first minced to defined particles of about 30 microns. After in situ gel digestion, the gel slurry is transferred into a mini reversed-phase column-funnel assembly in the sample loading station of the Hewlett-Packard protein sequencer. Applying nitrogen pressure elutes peptides from the gel slurry onto the reversed-phase material. The mini reversed-phase column is then placed in an in-line column adapter and connected to a micropreparative high performance liquid chromatography (HPLC) column, where separation of the peptides under standard conditions is achieved. In the work described here complete digestions and excellent peptide recoveries allowed the generation of extensive internal sequence information from low picomole amounts of proteins. The method has been routinely applied in both laboratories for two years.

L23 ANSWER 9 OF 19 MEDLINE on STN
AN 95379583 MEDLINE
TI Tertiary structure-selective characterization of protein dithiol groups by phenylarsine oxide modification and mass spectrometric peptide mapping.
AU Kussmann M; Przybylski M
SO METHODS IN ENZYMOLOGY, (1995) 251 430-5.
Journal code: 0212271. ISSN: 0076-6879.

L23 ANSWER 10 OF 19 MEDLINE on STN
AN 95379531 MEDLINE
TI High-resolution structural determination of protein-linked acyl groups.
AU Neubert T A; Johnson R S
SO METHODS IN ENZYMOLOGY, (1995) 250 487-94.
Journal code: 0212271. ISSN: 0076-6879.

L23 ANSWER 11 OF 19 MEDLINE on STN
AN 95143288 MEDLINE
TI Structural characterization of synthetic model peptides of the DNA-binding cI434 repressor by electrospray ionization and fast atom bombardment mass spectrometry.
AU Percipalle P; Saletti R; Pongor S; Foti S; Tossi A; Fisichella S
SO BIOLOGICAL MASS SPECTROMETRY, (1994 Dec) 23 (12) 727-33.
Journal code: 9102982. ISSN: 1052-9306.
AB The structural characterization of two synthetic model peptides of the cI434 repressor is described. Unequivocal determination of the structure was achieved by means of electrospray ionization mass spectrometry of the intact peptides and by fast atom bombardment mass spectrometric identification of complementary peptide fragments obtained by tryptic and chymotrypic digestion and partial separation by reversed-phase high-performance liquid chromatography. The results show the potential of this approach for characterizing synthetic peptides of relatively high molecular weight.

L23 ANSWER 12 OF 19 MEDLINE on STN
AN 94357883 MEDLINE
TI A mass spectrometric study on the in vivo posttranslational modification of GAP-43.
AU Taniguchi H; Suzuki M; Manenti S; Titani K

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Sep 9) 269 (36) 22481-4.
Journal code: 2985121R. ISSN: 0021-9258.

AB GAP-43 isolated from calf brain was analyzed by the electrospray mass spectrometry. The mass spectrum of the intact protein showed two species with a mass difference of 80 Da, suggesting that the isolated GAP-43 contains phosphorylated species. To establish the in vivo phosphorylation sites, the protein was digested with trypsin, and analyzed by the liquid chromatography/mass spectrometry technique, in which a capillary reversed-phase chromatography column was connected on line to an electrospray mass spectrometer. Two pairs of peptides with a mass difference of 80 Da were observed. From the tandem mass spectrometry, two novel phosphorylation sites (Thr-87 and Ser-152) were identified. The novel phosphorylation sites contain proline immediately after the phosphorylated serines. No phosphorylated peptide was detected corresponding to the protein kinase C or casein kinase II phosphorylation sites. A peptide corresponding to the acetylated N-terminal peptide was also identified. The mass of the peptide suggests that the 2 cysteinyl residues are not palmitoylated but form a disulfide bridge.

L23 ANSWER 13 OF 19 MEDLINE on STN
AN 94042989 MEDLINE
TI Processing and characterization of human proguanylin expressed in Escherichia coli.
AU Garcia K C; de Sauvage F J; Struble M; Henzel W; Reilly D; Goeddel D V
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Oct 25) 268 (30) 22397-401.
Journal code: 2985121R. ISSN: 0021-9258.

AB Guanylin is a 15-amino acid peptide hormone that was originally isolated from the jejunum of the rat small intestine and shown to be an endogenous activator of the intestinal heat-stable enterotoxin receptor-guanylyl cyclase. Guanylin is synthesized as a 115-amino acid prohormone, proguanylin, which is processed at a site yet to be determined, into a C-terminal bioactive fragment(s). In order to examine the processing of proguanylin in vitro, we have generated large quantities of the properly folded prohormone by constructing an expression vector that directs its secretion into the periplasmic space of Escherichia coli. The bacterially expressed human proguanylin was then processed to smaller C-terminal fragments by protease digestion. Digestion with trypsin or lysine-C generated C-terminal peptides of different length, which have been purified and characterized. Guanylin-22 and guanylin-32 have binding affinities and biological activities similar to guanylin-15, while guanylin-63 and the entire proguanylin have only minimal bioactivity. Circular dichroism spectroscopy reveals that proguanylin is a stably folded protein containing mostly beta-sheet and beta-turn structure.

L23 ANSWER 14 OF 19 MEDLINE on STN
AN 94004471 MEDLINE
TI Analytical and micropreparative peptide mapping by high performance liquid chromatography/electrospray mass spectrometry of proteins purified by gel electrophoresis.
AU Hess D; Covey T C; Winz R; Brownsey R W; Aebersold R
SO PROTEIN SCIENCE, (1993 Aug) 2 (8) 1342-51.
Journal code: 9211750. ISSN: 0961-8368.

AB We report the use of microbore reverse-phase high performance liquid chromatography connected on-line to an electrospray mass

spectrometer for the separation/detection of peptides derived by proteolytic digestion of proteins separated by polyacrylamide gel electrophoresis. A small fraction (typically 10% of the total) of the peptides eluting from the column was diverted through a flow-splitting device into the ion source of the mass spectrometer, whereas the majority of the peptide samples was collected for further analyses. We demonstrate the feasibility of obtaining reproducible peptide maps from submicrogram amounts of protein applied to the gel and good correlation of the signal detected by the mass spectrometer with peptide detection by UV absorbance. Furthermore, independently verifiable peptide masses were determined from subpicomole amounts of peptides directed into the mass spectrometer. The method was used to analyze the 265-kDa and the 280-kDa isoforms of the enzyme acetyl-CoA carboxylase isolated from rat liver. The results provide compelling evidence that the two enzyme isoforms are translation products of different genes and suggest that these approaches may be of general utility in the definitive comparison of protein isoforms. We furthermore illustrate that knowledge of peptide masses as determined by this technique provides a major advantage for error-free data interpretation in chemical high-sensitivity peptide sequence analysis.

L23 ANSWER 15 OF 19 MEDLINE on STN
AN 93055148 MEDLINE
TI Affinity purification of proteinases by a combination of immobilized peptidyl aldehyde and semicarbazone.
AU Basak A; Yuan X W; Seidah N G; Chretien M; Lazure C
SO JOURNAL OF CHROMATOGRAPHY, (1992 Oct 2) 581 (1) 17-29.
Journal code: 0427043. ISSN: 0021-9673.
AB D-Phe-Phe-argininal semicarbazone and Tyr-Gly-Gly-Phe-Leu-Arg-argininal semicarbazone were prepared using the solution phase synthesis method and characterized by mass spectrometry and nuclear magnetic resonance spectroscopy. The tripeptide and heptapeptide semicarbazones were individually immobilized on affi-Gel 15 resulting in two affinity columns called S3 and S7, respectively. A third affinity column was obtained by hydrolysing the semicarbazone moiety in column S3 to aldehyde (column A3). Serine proteinases such as trypsin or rat plasma kallikrein almost quantitatively bind to either S3 or A3 affinity columns. Under optimized conditions, more than 97% of trypsin bound to both columns S3 and A3. At a lower ionic strength and higher pH, 80-85% of rat plasma kallikrein bound to the same columns. Elution of both enzymes was achieved using mild conditions at near neutral pH and in the presence of a small amount of denaturant. Both proteinases were identified and characterized by high-performance liquid chromatography, sodium dodecylsulphate polyacrylamide gel electrophoresis and by their substrate specificity and inhibition profiles. A single purification (six-to seven-fold) step using either column S3 or A3 allowed the preparation of pure trypsin from commercial sources. Starting from rat plasma partially purified by a phenyl boronate column, fractionation on the S3 column allowed approximately an 87-fold purification of rat plasma kallikrein. However, serial purification of rat plasma kallikrein on column S7 followed by column A3 resulted in a purification factor of about 455.

L23 ANSWER 16 OF 19 MEDLINE on STN
AN 92176327 MEDLINE

TI Capillary electrophoresis-atmospheric pressure ionization mass spectrometry for the characterization of peptides. Instrumental considerations for mass spectrometric detection.
AU Johansson I M; Huang E C; Henion J D; Zweigenbaum J
SO JOURNAL OF CHROMATOGRAPHY, (1991 Aug 21) 554 (1-2) 311-27. Journal code: 0427043. ISSN: 0021-9673.
AB On-line capillary electrophoresis (CE) separations are shown for a synthetic peptide mixture and a tryptic digest of human hemoglobin in an uncoated fused-silica capillary with detection using atmospheric pressure ionization mass spectrometry (API-MS). The CE system utilized a 1-m capillary column of either 75- or 100-microns I.D. These somewhat larger inside diameters allow higher sample capacities for MS detection and the 1-m length facilitates connecting the CE column to the liquid junction-ion spray interface and MS system. Low volatile buffer concentrations (15-20 mM) of ammonium acetate or ammonium formate, and high organic modifier content (5-50%) of methanol or acetonitrile facilitates ionization under electrospray conditions. This study shows that peptides separated by CE may be transferred to the API-MS system through a liquid junction coupling to the pneumatically assisted electrospray (ion spray) interface at low buffer pH when the electroosmotic flow is low (0-0.04 microliter/min). CE-MS as described herein is facilitated by features in modern CE instrumentation including robotic cleaning and pressurization of the capillary inlet. The latter is particularly useful for repetitive rinsing and conditioning of the capillary column between analyses in addition to continuous 'infusion' of sample to the mass spectrometer for tuning purposes. In addition to facile molecular weight determination, amino acid sequence information for peptides may be obtained by utilizing on-line tandem MS. After the tryptic digest sample components enter the API-MS system, the molecular ion species of individual peptides may be focussed and transmitted into the collision cell of the tandem triple quadrupole mass spectrometer. Collision-induced dissociation of protonated peptide molecules yielded structural information for their characterization following injection of 10 pmol of a tryptic digest from human hemoglobin.

L23 ANSWER 17 OF 19 MEDLINE on STN
AN 91163438 MEDLINE
TI Peptides and proteins: overview and strategy.
AU Biemann K
SO METHODS IN ENZYMOLOGY, (1990) 193 351-60. Ref: 28 Journal code: 0212271. ISSN: 0076-6879.

L23 ANSWER 18 OF 19 MEDLINE on STN
AN 90062176 MEDLINE
TI Glutaredoxin from rabbit bone marrow. Purification, characterization, and amino acid sequence determined by tandem mass spectrometry.
AU Hopper S; Johnson R S; Vath J E; Biemann K
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Dec 5) 264 (34) 20438-47. Journal code: 2985121R. ISSN: 0021-9258.
AB A glutaredoxin was purified from rabbit bone marrow, and its amino acid sequence was determined by high performance tandem mass spectrometry. The sequences of peptides generated by digestion with trypsin alone or in combination with thermolysin were determined from their collision-induced dissociation (CID) mass spectra. Alignment of these sequences and additional sequence information

were obtained from the collision-induced dissociation mass spectra of peptides obtained from digestion of the intact protein with Staphylococcus aureus V8 protease and alpha-chymotrypsin. The resulting sequence of 106 amino acids is as follows: Ac-Ala-Gln-Glu-Phe-Val-Asn-Ser-Lys-Ile-Gln-Pro-Gly-Lys-Val-Val-Val-Phe- Ile-Lys-Pro-Thr-Cys-Pro-Tyr-Cys-Arg-Lys-Thr-Gln-Glu-Ile-Leu-Ser-Glu-Leu- Pro-Phe - Lys-Gln-Gly-Leu-Leu-Glu-Phe- Val-Asp-Ile-Thr-Ala-Thr-Ser-Asp-Met-Ser-Glu-Ile- Gln-Asp-Tyr-Leu-Gln-Gln-Leu-Thr-Gly-Ala-Arg- Thr-Val-Pro-Arg-Val-Phe-Leu-Gly-Lys-Asp-Cys-Ile-Gly-Gly-Cys-Ser-Asp-Leu-Ile-Ala-Met-Gln-Glu-Lys-Gly-Glu-Leu-Leu-Ala-Arg-Leu-Lys-Glu-Met-Gly- Ala-Leu-Arg-Gln. This glutaredoxin strongly resembles the corresponding calf and pig proteins (known as glutaredoxin and thioltransferase, respectively) with respect to its primary structure and enzymatic activity as a GSH:disulfide thioltransferase, an activity also found for the glutaredoxin from Escherichia coli. However, rabbit glutaredoxin was not active as a hydrogen donor for the reduction of ribonucleotides in the presence of the ribonucleotide reductases from rabbit bone marrow, Lactobacillus leichmannii, and Corynebacterium nephridii.

L23 ANSWER 19 OF 19 MEDLINE on STN
AN 87270754 MEDLINE
TI Microbore HPLC/mass spectrometry for the analysis of peptide mixtures using a continuous flow interface.
AU Caprioli R M; DaGue B; Fan T; Moore W T
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1987 Jul 15) 146 (1) 291-9.
Journal code: 0372516. ISSN: 0006-291X.
AB Microbore HPLC techniques have been combined with fast atom bombardment mass spectrometry to provide HPLC/MS capabilities for the analysis of mixtures of peptides and small proteins. The interface between the liquid chromatograph and mass spectrometer is a continuous flow direct insertion probe which contains a fused silica capillary that delivers the eluting solvent to the FAB source of the mass spectrometer at a rate of 5-10 microL/min. Data are presented for the analysis of several mixtures of peptides ranging in molecular weights from about 900 to 6000 daltons. In addition, the analysis of 100 pmol of a tryptic digest of whale myoglobin is shown where 16 of the possible 19 peptides were identified in the mass range m/z 2200-250. The advantages of this approach to HPLC/MS are a relatively high sensitivity because of the low flow rates and low background, and the ability to detect high molecular weight compounds.

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